Maarit Vaalamo

MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN NORMAL AND ABERRANT WOUND REPAIR

Expression patterns of collagenases-1 and -3, stromelysins-1 and -2, matrilysin, metalloelastase and TIMPs-1, -2, -3 and -4 in healing cutaneous wounds and in chronic ulcers of the skin and the intestine

University of Helsinki
Helsinki 2000

From the Department of Dermatology and Venereology,
Helsinki University Central Hospital, Helsinki, Finland

MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN NORMAL AND ABERRANT WOUND REPAIR

Expression patterns of collagenases-1 and -3, stromelysins-1 and -2, matrilysin, metalloelastase and TIMPs-1, -2, -3 and -4 in healing cutaneous wounds and in chronic ulcers of the skin and the intestine

by

Maarit Vaalamo

Academic Dissertation

To be presented, with the assent of the Medical Faculty of the University of Helsinki, for public examination in the Lecture Room of the Department of Dermatology and Venereology, Helsinki University Central Hospital, Helsinki, on February 4th, at 12 o`clock noon.

Helsinki 2000

Supervised by

Docent Ulpu Saarialho-Kere
Department of Dermatology and Venereology
Helsinki University Central Hospital, Finland

Reviewed by

Professor Aarne Oikarinen
Department of Dermatology
University of Oulu, Finland

and

Docent Matti Laato
Department of Surgery
University of Turku, Finland

Opponent

Professor Jyrki Heino
Department of Biological and Environmental Sciences
University of Jyväskylä, Finland

ISBN 951-45-8992-0 PDF version)

Helsingin yliopiston verkkojulkaisut
Helsinki 2000

To Mikko, Emilia and Veikka

CONTENTS

1. List of original publications 7
2. Abbreviations 8
3. Abstract 9
4. Introduction 10
5. Review of the literature 11
5.1. Structure of the skin and gut 11
5.1.1. Epithelium 11
5.1.2. Basement membrane 12
5.1.3. Connective tissue 13
5.2. Wound healing 16
5.2.1. Cutaneous wound healing 16
5.2.2. Intestinal wound healing 19
5.3. Chronic cutaneous wounds 20
5.4. Inflammatory bowel disease 21
5.5. Matrix degrading enzymes 21
5.5.1. Matrix metalloproteinases 22
5.5.1.1. Collagenases 22
5.5.1.2. Gelatinases 24
5.5.1.3. Stromelysins 24
5.5.1.4. Membrane-type metalloproteinases 25
5.5.1.5. Other matrix metalloproteinases 26
5.5.2. Regulation of the matrix metalloproteinases 26
5.5.2.1. Regulation at the transcriptional level 26
5.5.2.2. Zymogen activation 27
5.5.2.3. Inhibition of the activity of metallo-proteinases 28
5.5.2.4. Tissue inhibitors of metalloproteinases (TIMPs) 28
5.6. Matrix metalloproteinases in cutaneous wound repair 29
5.7. Matrix metalloproteinases in intestinal disease 32
5.8. Matrix metalloproteinases in wound repair in other tissues 32
5.9. Matrix metalloproteinases as targets of wound therapies 33
6. Aims of the study 34
7. Materials and methods 35
7.1. Tissue samples 35
7.2. RNA probes 36
7.3. *In situ* hybridization 37
7.4. Immunohistochemistry 37
7.5. Cell cultures 38
7.6. Northern blot analysis 39
8. Results 40
8.1. Cutaneous wounds (II, III, V) 40
8.2. Intestinal ulcerations (I, IV) 42
8.3. Experimental murine intestinal anastomoses (IV) 44
8.4. Experimental piglet wounds (II) 44

8.5. Human skin fibroblast culture (III) 44
9. Discussion 45
9.1. Collagenases-1 and –3 and stromelysins-1 and –2 in cutaneous wound healing (I, III) 45
9.1.Tissue inhibitors of matrix metalloproteinases-1, -2, -3 and –4 in cutaneous wound healing (II, V) 49
9.2. Collagenases-1 and –3, stromelysins-1 and –2, matrilysin, and macrophage metalloelastase in inflammatory bowel diseases (I, IV) 50
9.2. Tissue inhibitors of matrix metalloproteinases-1 and -3 in inflammatory bowel diseases (I, IV) 53
9.5. Matrix metalloproteinases in animal models (II, IV) 53
10. Conclusions 55
11. Acknowledgements 56
12. References 58

1. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Saarialho-Kere U, Vaalamo M, Puolakkainen P, Airola K, Parks WC, and Karjalainen-Lindsberg M-L: Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. Am J Pathol 1996, 148: 519-526

II Vaalamo M, Weckroth M, Puolakkainen P, Saarinen P, Kere J, Lauharanta J, and Saarialho-Kere U: Patterns of matrix metalloproteinase and TIMP-1 expression in chronic and normally healing cutaneous wounds. Br J Dermatol 1996, 135:52-59

III Vaalamo M, Mattila L, Johansson N, Karjalainen-Lindsberg M-L, Kariniemi A-L, Kähäri V-M and Saarialho-Kere U: Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds. J Invest Dermatol 1997, 109: 96-101

IV Vaalamo M, Karjalainen-Lindsberg M-L, Puolakkainen P, Kere J, Saarialho-Kere U: Distinct expression profiles of stromelysin-2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (MMP-12) and tissue inhibitor of metalloproteinases-3 (TIMP-3) in intestinal ulcerations. Am J Pathol 1998, 152: 1005-1014

V Vaalamo M, Leivo T, Saarialho-Kere U: Differential expression of tissue inhibitors of metalloproteinases (TIMP-1, -2, -3 and -4) in normal and aberrant wound healing. Human Pathol 1999, 30: 795-802

The publications are referred to in the text by their Roman numerals

2. ABBREVIATIONS

AP-1	activating protein-1
BPAG	bullous pemphigoid antigen
bFGF	basic fibroblast growth factor
BM	basement membrane
cDNA	complementary deoxyribonucleic acid
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EGF	epidermal growth factor
ETS	E26 transformation specific
HD-1	hemidesmosomal protein-1
IBD	inflammatory bowel disease
IFAP300	intermediate filament associated protein 300
IGFBP	insulin-like growth factor binding protein
IL	interleukin
kDa	kilodalton
KGF	keratinocyte growth factor
MBP	myelin basic protein
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
MT-MMP	membrane-type metalloproteinase
PA	plasminogen activator
PEA-3	polyoma enhancer activator-3
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PMA	phorbol 12-myristate 13-acetate
RNA	ribonucleic acid
SPARC	secreted protein acidic and rich in cysteine
TGF-α	transforming growth factor-alfa
TGF-β	transforming growth factor-beta
TIMP	tissue inhibitor of metalloproteinases
TNF-α	tumor necrosis factor-alfa
tPA	tissue-type plasminogen activator
uPA	urokinase-type plasminogen activator
VEGF	vascular endothelial growth factor

3. ABSTRACT

Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes, that are collectively able to degrade most, if not all, components of the extracellular matrix (ECM). This capacity is needed in conditions with active remodeling of the connective tissue, such as fetal development, cancer invasion and metastasis, as well as wound healing. In this study, the expression patterns of MMPs and tissue inhibitors of metalloproteinases (TIMPs) were investigated in normally healing and chronic cutaneous wounds, and in chronic intestinal ulcerations. The principal methods used were *in situ* hybridization and immunohistochemistry.

In normally healing cutaneous wounds, the migrating front of keratinocytes expressed collagenase-1 and stromelysin-2. These enzymes were induced within 1-3 days after wounding, and the expression was turned off after complete re-epithelialization. Stromelysin-1 was expressed by proliferative keratinocytes on a newly formed basement membrane. Collagenase-1 and stromelysins-1 and -2 were expressed in the same spatial pattern also in the epithelium of chronic ulcers. In addition to epithelial events, MMPs were involved in stromal remodeling. In both normally healing and chronic wounds, the expression of collagenase-1 by stromal cells was a constant finding. Contrasting this, collagenase-3 was only expressed in fibrotic areas of chronic ulcers, but not in acute wounds. Stromelysin-1 mRNA was detected in the stroma in both acute and chronic wound samples.

TIMPs-1 and –3 were expressed by basal, proliferating keratinocytes in normally healing, but not in chronic cutaneous wounds, suggesting imbalance of the MMPs and their inhibitors in chronic ulcers. TIMPs-1 and -3 were also expressed by stromal cells in both wound types. TIMP-2 protein was detected in wound stroma, and particularly in acute wounds, surrounding the migrating front of keratinocytes. TIMP-4 protein was only found in few stromal cells in chronic ulcers.

No collagenase-1 nor stromelysin-1 mRNAs were detected in intestinal epithelium. Instead, matrilysin and stromelysin-2 were expressed by migrating intestinal epithelial cells. Collagenase-1, collagenase-3 and stromelysin-1 were abundantly expressed by activated fibroblast-like cells beneath erosions or ulcerations in inflammatory bowel disease (IBD). Macrophage metalloelastase mRNA was detected in macrophages within the inflammatory infiltrate, and underneath the shedding epithelium. TIMPs-1 and –3 mRNAs were detected in the stroma of IBD lesions as well, but, as in chronic cutaneous wounds, the epithelium remained negative.

In conclusion, successful wound healing is accompanied by tightly scheduled expression of metalloproteinases and their inhibitors. Their imbalance may delay wound healing and result in chronic ulcers. MMPs and TIMPs are also involved in both tissue destruction and mucosal reparative processes during the course of inflammatory bowel diseases.

4. INTRODUCTION

Wound healing is a process that results in restoration of the injured tissue. It involves re-epithelialization, granulation tissue formation and connective tissue remodeling. These events require controlled lysis of the extracellular matrix, important mediators of which are matrix metalloproteinases (matrixins, MMPs). MMPs comprise a group of enzymes that collectively are able to degrade most, if not all, components of the extracellular matrix (ECM). These enzymes are divided into subfamilies of collagenases, stromelysins, gelatinases, and MT-MMPs, based on their structure and substrate specificities. The expression of MMPs is controlled by cytokines, growth factors, oncogenes, and changes in the cell-cell and cell-matrix interactions. In the skin, the main cell types expressing these enzymes are keratinocytes, fibroblasts, macrophages, and endothelial cells. Tissue inhibitors of metalloproteinases (TIMPs) -1, -2, -3 and -4, are the most important inhibitors of MMPs, whose activities are required not only to prevent excessive proteolysis of tissues but also to regulate e.g. cell proliferation and angiogenesis. MMPs, controlled by their inhibitors, are involved in physiological processes such as reproduction and fetal development. An imbalance between MMPs and TIMPs has been implicated in abnormal tissue degradation leading to chronic wounds as well as cancer invasion and metastasis.

In this study, we investigated the cellular origin, and the temporal expression patterns of collagenases-1 and –3, stromelysins-1 and –2, matrilysin and macrophage metalloelastase in normally healing and chronic wounds of the skin. Furthermore, MMP-expression profiles in chronic, erosive and/or ulcerative lesions caused by inflammatory bowel disease (IBD), multifactorial disorders of the intestine, were studied. The inhibitors of MMPs, TIMPs, as well as various ECM proteins, were also examined in lesions of the skin and the intestine.

5. REVIEW OF THE LITERATURE

5.1. STRUCTURE OF THE SKIN AND GUT

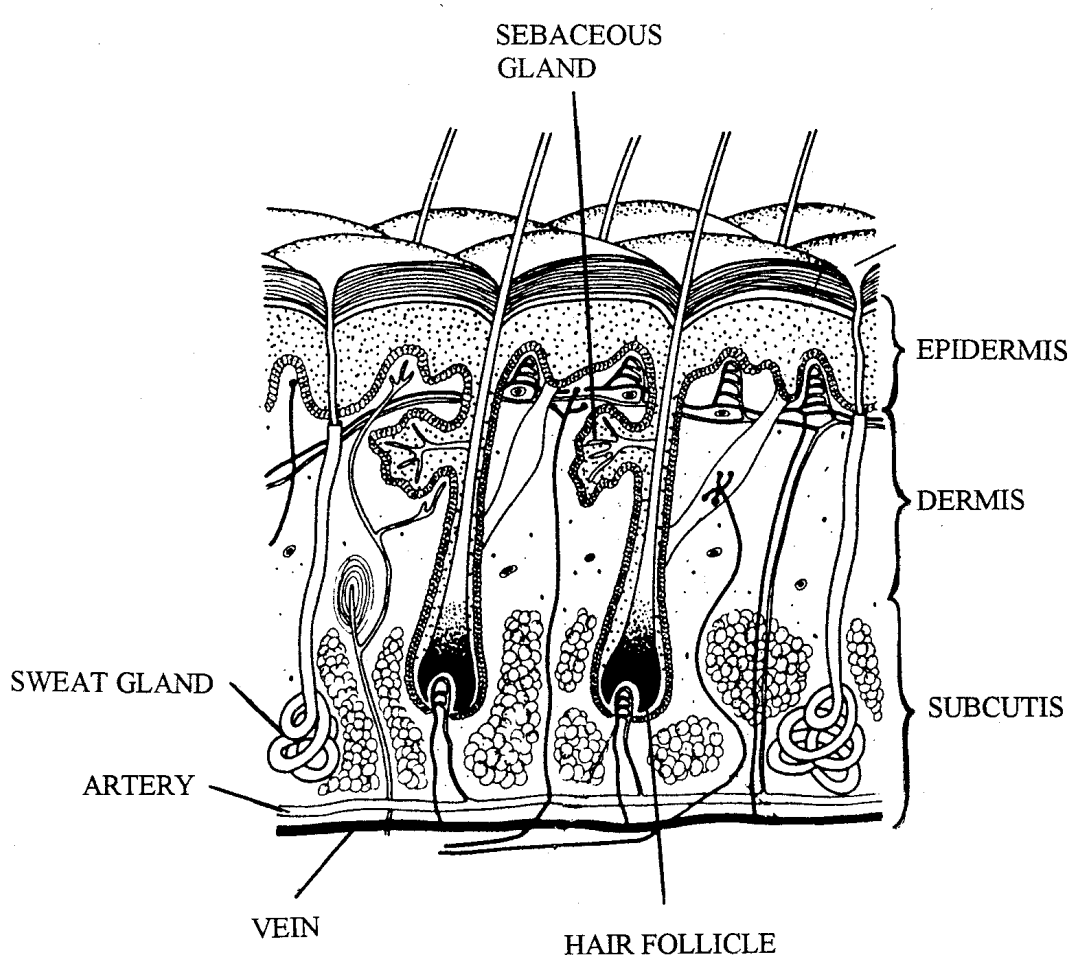

Figure 1. Structure of the skin. Modified from Kariniemi & Kauppinen, 1989

5.1.1. Epithelium

Epithelium is comprised of one or multiple layers of cells, that cover the exterior surfaces of the body and line the internal body cavities and tubes. It also forms the secretory portion of the glands and their ducts. The epithelium is multifunctional: an almost impermeable barrier as in the skin, secretory as in the glands, or both secretory and absorptive as in the intestine.

Skin:
The stratified epithelial layer of the skin, the epidermis, forms a barrier to protect the individual from water loss, entry of micro-organisms, and radiation. The main cell type of the epidermis is the keratinocyte, which migrates from the basal layer outwards, and is ultimately sloughed from the skin surface. Other cell types found in the epidermis are Langerhans cells, which have immunologic

functions, melanocytes, which donate pigment to the keratinocytes, and Merkel cells. The layers of epidermis differ from each other by the state of keratinocyte differentiation. In the basal cell layer undifferentiated, proliferating cells lie on an intact basement membrane. Spinous layer and granular layer are named after their microscopic appearance. Keratinocytes of the spinous layer synthesize keratin peptides, while the cells in the granular layer contain granules of profilaggrin and lipid. The transition zone where the cornified envelope is constructed, forms a region between the living and the dead cells. The cornified cell marks the final point of keratinocyte differentiation. Almost 80% of the corneocytes is keratin, but also remnants of organelles, melanin and membrane profiles are found. (see Eckert et al, 1989; see Eady et al, 1998)

Intestine:
Unlike the epidermis, the epithelium of the intestinal tract is formed by a single layer of columnar cells, that form glands, pits, villi or crypts, depending on the region of the gut. Five cell types are present in the intestinal epithelium, namely absorptive cells enterocytes, mucus producing goblet cells, secretory APUD cells, Paneth cells, and undifferentiated cells. Every 3-8 days, the epithelium is renewed. This process begins in the proliferative zone, where the undifferentiated epithelial cells differentiate and migrate towards the luminal surface to be sloughed off. (see Ross & Reith, 1985a; see Graham et al, 1992).

5.1.2. Basement membrane

Skin & Intestine:
Basement membranes are continuous sheets that separate layers of cells from the underlying connective tissue. They are structurally rather similar in different organs. Basement membranes provide means of support for cell layers, regulate cell attachment, growth and differentiation. They also act as molecular sieves between tissue compartments, and are able to select certain cells for passage through the BM. (see Yurchenko & Schnitty, 1990)

Basement membranes are formed from components produced by both epithelial and stromal cells. These components include the network-forming type IV collagen and laminin-1, that are found in a variety of basement membranes, including those under skin and intestinal epithelium (see van der Rest & Garrone, 1991; Foidart et al, 1980; Grant & Leblond, 1988). In the small intestine, however, laminin-1 and -2 are expressed reciprocally, the former as the villus-form, and the latter as the crypt-form (see Beaulieu, 1999). Nidogen/entactin links type IV collagen and laminin-1 fibers and anchors other molecules of the BM. Other components of the BM include heparan sulphate proteoglycans such as perlecan that confer filtrating properties, SPARC (secreted protein acidic and rich in cysteine) that appears essential during development and wound healing, and fibulins. (see Yurchenko & Schnitty 1990; Yurchenko & O`Rear 1994; Uitto et al, 1995)

The hemidesmosomes are specialized junctional complexes, that contribute to epithelial cell attachment to the underlying BM in epithelia of the skin, the cornea, parts of gastrointestinal and respiratory tract, and the amnion. They are composed of plectin (McLean et al, 1996), bullous pemphigoid antigen (BPAG) 1 (Stanley et al, 1988), HD1, intermediate filament associated protein (IFAP) 300, bullous pemphigoid antigen 2 (BPAG2, type XVII collagen) (Giudice et al, 1992), and $\alpha_6\beta_4$ integrin (Stepp et al, 1990; Sonnenberg et al, 1991). Plectin and BPAG1 form the cytoplasmic

plaque together with two less well characterized proteins called HD1 and IFAP 300 (see Borradori & Sonnenberg, 1999). The β4 integrin subunit binds to plectin, which mediates the interaction between the hemidesmosome and the keratin cytoskeleton. Laminin-5 (Rousselle et al, 1991) binds the transmembrane constituents $\alpha_6\beta_4$ integrin, and possibly also BPAG2, and traverses through lamina lucida to the lower portion of the BM. The connection to underlying stroma is mediated by laminin-5 attachment to type VII collagen, which forms a component of anchoring fibrils and extends to the papillary dermis. (see Burgeson & Christiano 1997; see Borradori & Sonnenberg, 1999)

The anchoring system of intestinal epithelial cells differs from that of the skin by the electron microscopic absence of hemidesmosomes (Leivo et al, 1996). In the intestine, adhesion complex components laminin-5 and type VII collagen are confined to surface epithelia, suggesting some sort of an adhesion complex, while the epithelium of the crypts lacks these components (Leivo et al, 1996; Lohi et al, 1996; Orian-Rousseau et al, 1996).

5.1.3. Connective tissue

The connective tissue is a supporting matrix, which also directs tissue development and interacts with connective tissue cells. It is divided into two general subtypes. The loose connective tissue contains aggregates of loosely arranged fibers and different cells, many of which participate in the defense mechanism of the body. The dense connective tissue contains more numerous and thicker fibers, but considerably fewer cells of chiefly one type, the fibroblast.

Connective tissue components
Fibrillar collagens types I and III, and type II in the bone and cartilage, are the most abundant components of the connective tissue. Most of the collagens are synthesized by fibroblasts as procollagen molecules (Prockop & Kivirikko, 1995), which are then processed in the extracellular space. Nonfibrillar collagens form sheetlike structures, e.g. type IV collagen in the basement membranes, or connect ECM components to collagen fibrils. (see van der Rest & Garrone, 1991)

The elasticity of the connective tissue is provided by the elastic fibers, complex structures that contain their predominant protein elastin, microfibrillar proteins, and perhaps, proteoglycans. Elastin endows the fibers with the characteristic property of elastic recoil. (see Mecham & Heuser, 1991)

Proteoglycans form the amorphous ground substance of the connective tissue. They are complex molecules with a core protein and glycosaminoglycan side chains. Proteoglycans are found inside cells, on the cell surface, and in the ECM. They may influence cell proliferation and adhesion, have protease and antiprotease function, and act as polypeptide growth factors (see Wight et al, 1991; see Pope et al, 1998).

Fibronectin is a cell interactive protein present in a variety of extracellular matrices. It is produced by various cell types, and has the ability to bind to ECM macromolecules and to integrin receptors. It is upregulated during e.g. wound healing when it facilitates cell adhesion and migration. (see Yamada, 1991)

Tenascin, SPARC (secreted protein acidic and rich in cysteine) and thrombospondin are anti-adhesive ECM glycoproteins that modulate cell-matrix interactions. For example, tenascin inhibits cell adhesion and spreading, and promotes cell rounding, SPARC has been shown to induce MMP expression in fibroblasts, and thrombospondin promotes angiogenesis in vitro (Chiquet-Ehrismann et al, 1989; Tremble et al, 1993; Nicosia & Tuszynski, 1994). These proteins are present in normal ECM in faint amounts, but are greatly upregulated during morphogenesis and remodeling. Interestingly, wounds in tenascin-C deficient mice heal normally (Forsberg et al, 1996), and wounds in mice deficient in thrombospondin-2 appear to heal at an accelerated rate, but have an abnormal granulation tissue (Kyriakides et al, 1999). (see Sage & Bornstein, 1991; see Lightner, 1994).

Connective tissue cells
Fibroblasts are the main cell type found in the connective tissue. Fibroblasts are largely responsible for the production of extracellular fibers and the ground substance of connective tissue. *Myofibroblasts* contain relatively large amounts of myofilaments, but differ from *smooth muscle cells* by lacking the basal lamina. *Mast cells* occur in most tissues, but are particularly numerous in skin, bronchus, nasal mucosa and gut. They contain granules with varying amounts of neutral proteinases tryptase and chymase. *Macrophages* are cells of diverse functions, e.g. phagocytosis, antigen presenting, and secretion of growth factors and proteinases. *Plasma cells* are derivatives of B lymphocytes, which actively produce antibodies, and are especially numerous in the intestinal lamina propria. Transient, blood-derived cells include *neutrophils*, *eosinophils*, *monocytes* and *lymphocytes*. In addition, there may be *adipocytes* and *undifferentiated mesenchymal cells*. (See Pope, 1998; see Ross & Reith, 1985b)

Integrins
Integrins are a group of receptors by which cells attach to surrounding matrix or other cells. They are heterodimers consisting of α and β subunits, that traverse the cell membrane. At least 8 β- and 15 α-subunits form numerous combinations, currently more than 20, which are divided in subgroups based on their β-subunit (see Table 1.) The major ligands for integrins are 1) extracellular matrix proteins, e.g. collagens and fibronectin, and 2) cell surface molecules, e.g. intracellular adhesion molecules. The integrins can often bind more than one ligand, and ligands can in turn often recognize more than one integrin. The integrins do not act solely as a means of attachment between cells and the matrix components, but are also able to mediate signals through the cell membrane in either direction, and therefore regulate cell functions. (see Hynes, 1992; see Petruzelli et al, 1999; see Giancotti & Ruoslahti, 1999).

Normal epidermis has a characteristic distribution of the integrins. The $\alpha_6\beta_4$ integrin is a component of the hemidesmosome and mediates attachment of the basal keratinocytes to the basement membrane zone (see Yancey, 1995). $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins locate at the latero-apical membrane of the basal keratinocytes (Peltonen et al, 1989). In human small intestine, α_6 and β_1 subunits locate at the base of all enterocytes, while α_1, α_2, and α_3 subunits are expressed in distinctive crypt-villus gradients, reflecting the proliferation-migration-differentiation cascade of the intestinal epithelial cells (Beaulieu, 1992). The α_2 and α_3 subunits are expressed in basolateral aspects of the intestinal cell, the former being predominant in the crypts, and the latter on the villi (Beaulieau, 1992). β_4 subunit is found in the entire crypt-villus epithelium in the small intestine (see Beaulieau, 1999). However, the alpha-beta association of these interin subunits has not been investigated (see Beaulieau, 1999). In gastric mucosa, $\alpha_3\beta_1$ and $\alpha_6\beta_4$ integrins are expressed closely confined to BM areas in parts of gastric pits and surface epithelium, whereas the $\alpha_6\beta_1$ integrin is found throughout the gastric epithelium (Virtanen et al, 1995).

Table 1. Integrin ligands

Integrin		*Ligands*
β1	α1	collagens, laminin
	α2	collagens, laminin
	α3	fibronectin, laminin, collagens, laminin-5
	α4	fibronectin, VCAM-1
	α5	fibronectin
	α6	laminin
	α7	laminin
	α8	osteopontin, vitronectin, fibronectin
	α9	tenascin
	αV	fibronectin, osteopontin, vitronectin
β2	αL	ICAM-1, ICAM-2, ICAM-3
	αM	C3b, fibrinogen, factor X, ICAM-1
	αX	C3b, fibrinogen
β3	αIIb	fibronectin, fibrinogen, vWf, vitronectin, thrombospondin
	αV	osteopontin, collagens
β4	α6	laminin, laminin-5 (Niessen et al, 1994)
β5	αV	fibronectin, vitronectin
β6	αV	fibronectin, tenascin
β7	α4	fibronectin
	αE	E cadherin
β8	αV	fibronectin, VCAM-1, laminin

Modified from Petruzelli et al, 1999 and Clark, 1995. VCAM vascular cell adhesion molecule; ICAM intracellular adhesion molecule; vWF von Willebrandt factor.

Skin:

The connective tissue of the skin, the dermis, is divided into the subepithelial papillary dermis of the loose connective tissue (1/10), and the reticular layer of thick collagen bundles (9/10), which lies on adipose tissue. The papillary dermis contains a rich supply of blood vessels that penetrate from the deeper layers. Dermal matrix consists mainly (75% of its dry weight) of fibrillar type I collagen (>70%) and type III collagen (15%), which provide the skin with tensile strength. The reticular dermis contains the skin appendices hair follicles, eccrine and apocrine sweat glands, and sebaceous glands, ducts of which extend to the epidermis. (see Pope, 1998)

Intestine:

The intestine is divided in the following layers: 1) the mucosa, that consists of epithelium, basement membrane, lamina propria, and muscularis mucosa, 2) the submucosa, 3) the muscularis propria, and 4) the serosa which forms the outside cover of the intestine. The lamina propria is a loose connective tissue composed mainly of collagens types I, III, and V, and elastin. It has a network of capillaries and lymphatics and numerous mesenchymal as well as inflammatory cells, which act in challenging and destroying antigens and other foreign substances such as bacteria. The lamina propria is separated from the submucosa by a thin layer of smooth muscle cells named muscularis mucosa. The submucosa is a loose connective tissue with many vessels. The collagen content differs from that of the skin by a larger amount of type III (20 %) and type V (12%) collagens, while 68 % is type I collagen. Another characteristic feature is the activity of the smooth muscle cells in the maintenance of the extracellular matrix of the intestinal wall. (see Ross & Reith, 1985a; see Graham et al, 1992)

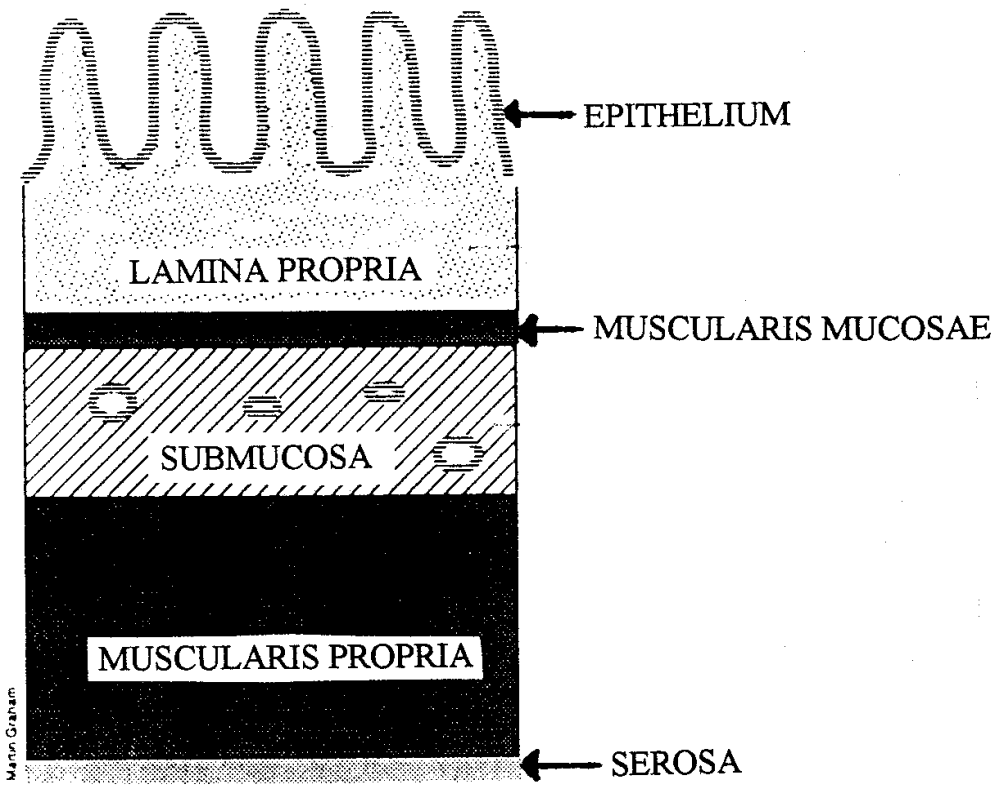

Figure 2. Layers of the intestinal wall. Modified from Graham et al, 1992.

5.2. WOUND HEALING

Wound healing is a complex process during which the injured tissue, e.g. skin or intestinal wall, is repaired. It involves inflammation, re-epithelialization, neoangiogenesis, and connective tissue cell activation with subsequent ECM degradation and resynthesis. All these processes are regulated by cell-ECM interactions and by various cytokines and growth factors. Injury initiates the rapid onset of a vigorous, multicellular wound healing reaction, which then gradually, during following weeks or months, proceeds towards a rather acellular scar.

5.2.1. Cutaneous wound healing

Re-epithelialization

Keratinocyte migration is needed for the wound to be recovered by the epithelium. Migration over the provisional matrix begins within hours after wounding. The migratory keratinocytes originate not only from the cut edge of the wound, but also from the skin appendages. Platelets release growth factors such as PDGF, TGF-β, and EGF-like growth factors from their α-granules, and initiate coagulation, which results in the formation of a provisional matrix. This matrix consists mainly of fibrin and fibronectin and provides a surface on which epidermal cells migrate (Clark et al, 1982). Wound area is rich in cytokines and growth factors, which may induce migration (see Slavin, 1996).

KGF and EGF, for example, are abundant in healing wounds, and induce keratinocyte migration and proliferation (Werner et al, 1992; Tsuboi et al, 1993; see Clark, 1995). An alternate stimulus for migration is provided by contact of the keratinocytes with fibronectin, fibrin and vitronectin via integrins. In wounded epidermis, integrins $\alpha_3\beta_1$, $\alpha_5\beta_1$, and $\alpha_v\beta_5$ appear on greater amounts on basal and suprabasal keratinocytes, possibly mediating migration over the provisional matrix (Kim et al, 1992; Larjava et al, 1993a; Cavani et al, 1993; Juhasz et al, 1993; Kim et al, 1994). While actively migrating, the keratinocytes produce the BM component laminin-5, and proteases e.g. collagenase-1 and uPA (Grøndahl-Hansen et al, 1988; Larjava et al, 1993a; Saarialho-Kere et al, 1993b). Proliferation of the wound edge keratinocytes begins within two days after injury, possibly induced by altered growth factor/growth factor receptor profiles. A relatively late event in human wound healing is the expression of fibronectin and tenascin binding integrin $\alpha_v\beta_6$ by the keratinocytes covering the wound bed (Haapasalmi et al, 1996). (see Clark, 1995; see Woodley, 1996)

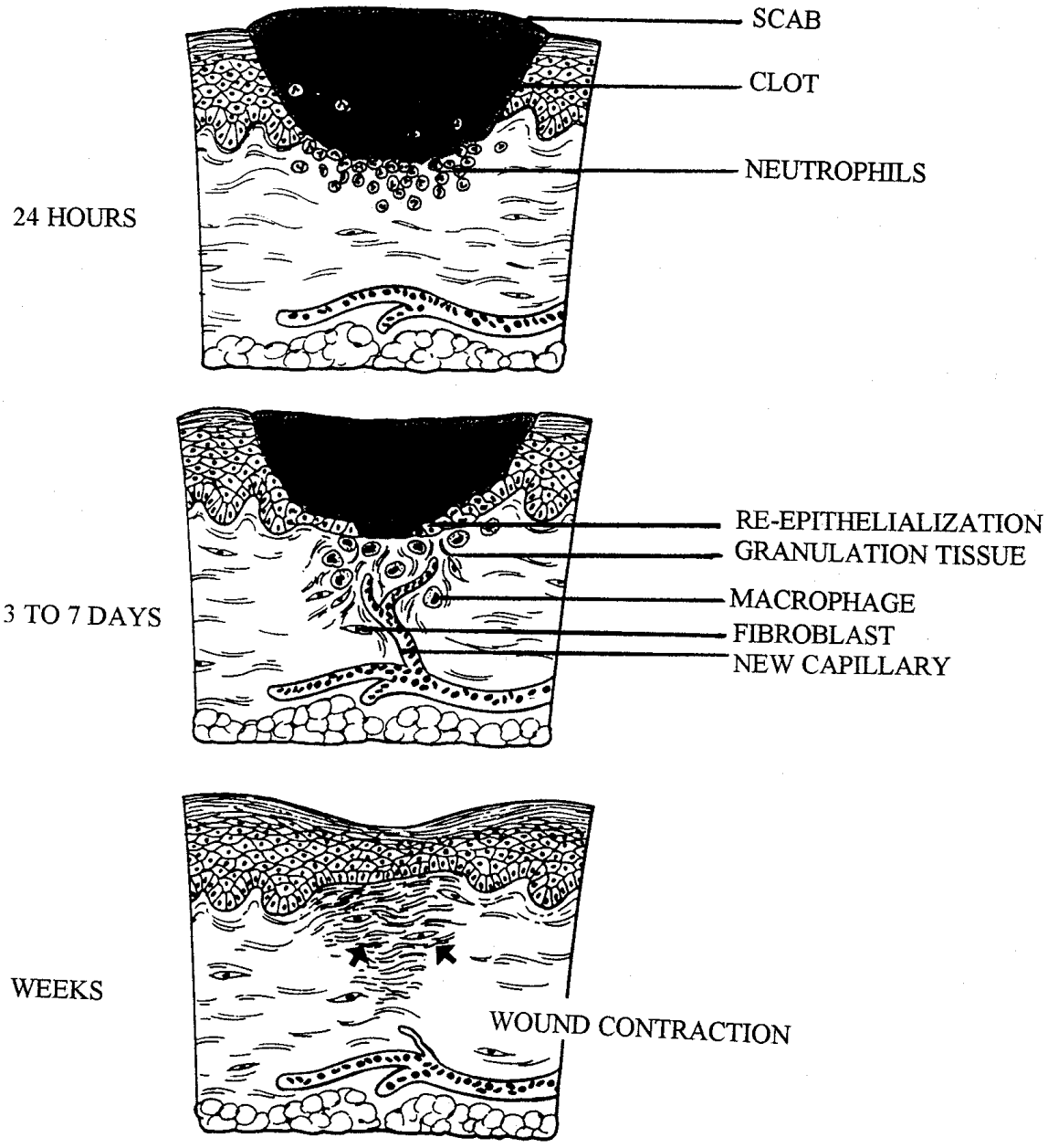

Figure 3. Dermal wound repair. Modified from Robbins et al, 1989a.

Stromal remodeling:

1. The inflammatory phase

The inflammatory phase of wound healing is characterized by increased vascular permeability, local release of cytokines and growth factors, and activation of migrating cells. Neutrophils are the first cells to arrive to clear the injured area from bacteria. Cytokines released by platelets act as chemotactic agents for neutrophils and macrophages, which in turn secrete e.g. TNF-α and IL-1, (see Steed, 1997; see Singer & Clark 1999). Influx and activation of macrophages is critical to the wound healing process. They participate in matrix synthesis and degradation, and are able to mediate angiogenesis and fibroplasia as well as activate e.g. lymphocytes via excretion of cytokines. (see Clark, 1995)

2. The proliferative phase

During the proliferative phase, fibroblast-like cells and endothelial cells become activated and migrate from surrounding tissues and closeby venules, respectively, to the wound area. The provisional matrix serves as a reservoire for various cytokines, directs signals to the cells via integrin receptors, and its collagen and fibronectin provide scaffolding for contact guidance for migrating cells. While migrating, the fibroblasts use integrins to attach to provisional matrix fibronectin and fibrinogen (Huhtala et al, 1995; Gailit & Clark, 1996). Mesenchymal cell activation appears to be the rate-limiting step of the beginning of granulation tissue formation around three days after injury (McClain et al, 1996). Their activation and chemotaxis is mediated mainly via cytokines such as PDGF and TGF-β, released from α-granules of platelets and produced by activated macrophages. After migration to the injured area, fibroblasts together with macrophages begin to produce proteins for the ECM, including glycosaminoglycans, proteoglycans, and other proteins such as SPARC, tenascin and thrombospondin. Newly formed blood vessels provide for the nutrient and oxygen supplies. VEGF together with bFGF may enhance neovascularization (see Slavin et al, 1996). Collagen synthesis by fibroblasts begins in the deep dermis during the first days after wounding (Scharffetter et al, 1989; Petri et al, 1997), possibly induced by TGF-β, which is abundantly expressed in wounds (Quaglino, 1990; Quaglino, 1991; see Raghow, 1991). Macrophage products TNF-α and IL-1 have an opposite effect and reduce collagen production by experimental granulation tissue fibroblasts (Rapala et al, 1996; Rapala, 1997; see Steed, 1997). Interestingly, low concentration of PDGF-AB up-regulates collagen synthesis, while high concentration down-regulates it (Lepistö et al, 1995). During the first days after wounding, collagen type III is the main fibrillar collagen produced, and is later gradually replaced by collagen type I. Transformation of the granulation tissue fibroblasts into myofibroblasts results in the contraction of the wound with subsequent diminishing of the wound area. This may be mediated by TGF-β, which has been shown to enhance contraction of collagen gels by dermal fibroblasts (Reed et al, 1994; Montesano et al, 1988). Furthermore, the involvement of integrin α2β1 in wound contraction has been suggested (Schiro et al, 1991). (see Gailit & Clark, 1994; see Clark, 1995; see Witte & Barbul, 1997)

3. The remodeling phase

Remodeling is the longest phase of wound healing, lasting from weeks to months. During this phase, the neovascularization recedes and the loose provisional matrix is gradually replaced by collagen fibers, which are thereafter rearranged by proteolysis and reproduction, to provide the scar with tensile strength. While the provisional matrix collagen is almost exclusively of type III, during the course of wound healing it is degraded by collagenase activity, and replaced by collagen type I, the main collagen of the mature scar. The maturation of the scar tissue is completed in about one year. Despite long-lasting remodeling of the matrix, the wound area never reaches the level of

organization of normal fibrillar collagens. The resulting scar tissue gains maximum 80 % of the tensile strength of normal skin. (see Clark, 1995; see Witte & Barbul, 1997; see Gailit & Clark, 1994)

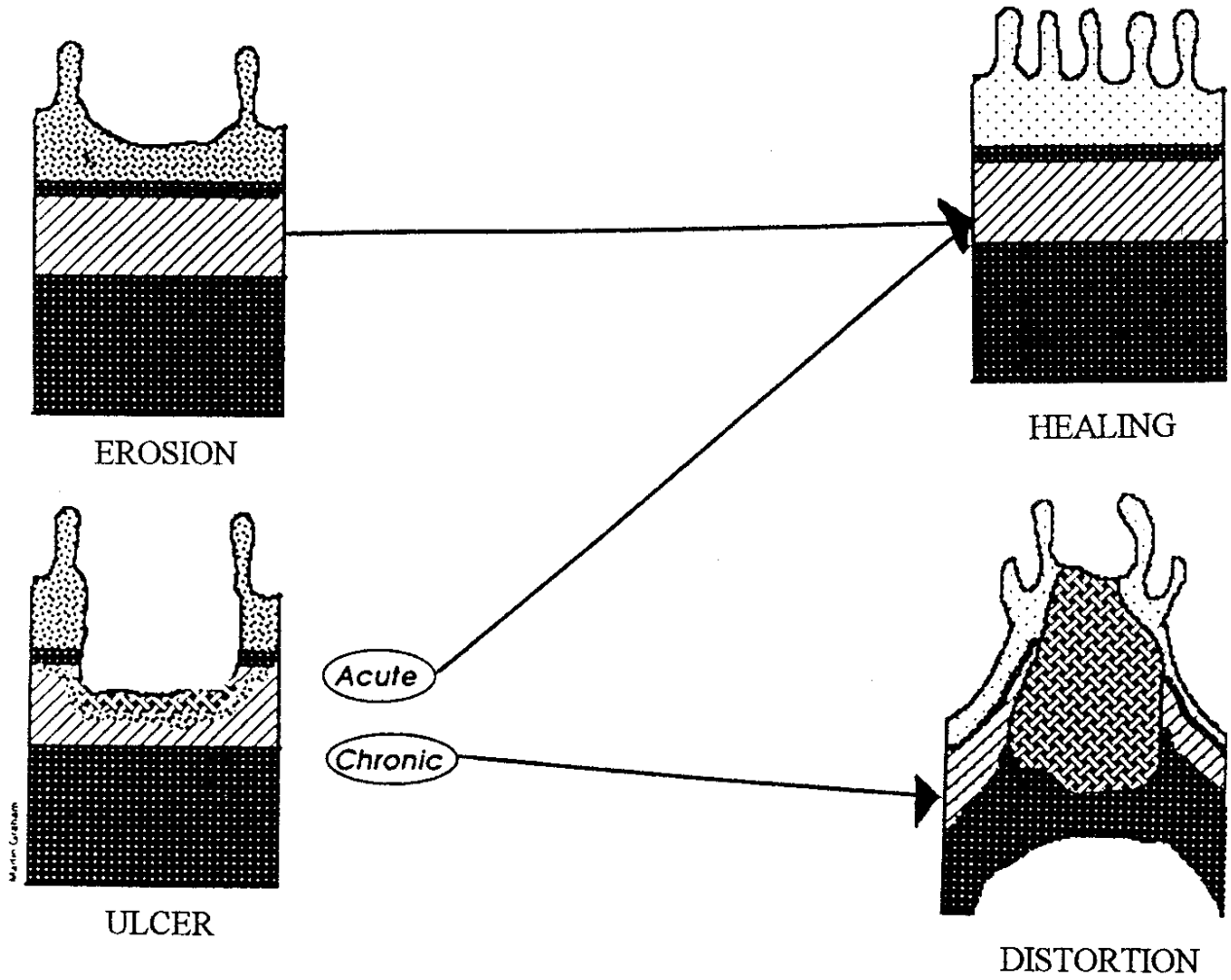

Figure 4. Intestinal wound repair. Modified from Graham et al, 1992.

5.2.2. Intestinal wound healing

In the intestine, the healing process depends on how deep the injury penetrates. 1) Superficial damage confined to the epithelial layer results in rapid covering of the wound by epithelial cells without inflammation and scar formation. This process called restitution begins shortly after injury by shedding of the damaged cells, which then form a cover for the restituting epithelium. The viable epithelial cells become flattened and start to migrate on the basement membrane until the cells touch and form new tight junctions. Restitution is completed within hours, and differs from normal epithelial migration by the lack of mitosis. (for review, see Lacy, 1988 and Wilson & Gibson, 1997) 2) Healing of mucosal lesions, erosions, involves inflammation and proliferation, but leaves no clinical or histological evidence of scar. Thus, the mucosa appears to heal itself by reconstitution and regeneration, and not by repair. 3) Healing of deeper lesions involving the submucosal layers of the intestine, intestinal ulcers, varies according to the duration of the lesion. Acute intestinal ulcers

often have minimal fibrotic response, and resorption of the scar tissue occurs. In chronic intestinal ulcers, there is significant fibrosis in the deeper layers of the intestine, which results in morphological changes such as stricture formation. 4) Surgical intervention results in incisional or anastomotic healing. This healing process is initiated by short-term, full-thickness injury to the intestine. It mimics that of healing dermal wounds with phases of hemostasis, inflammation, proliferation and remodeling. A major difference is the role of smooth muscle cells instead of fibroblasts in collagen production. Growth factors may, as well, serve varying roles in wound healing in different organs; TGF-β, for example, enhances collagen production in skin fibroblasts, but suppresses it in colonic fibroblasts (Martens et al, 1992). Growth factors such as EGF and TGF-α are vital in epithelial migration, while bFGF and VEGF stimulate angiogenesis during intestinal wound healing (see Jones et al, 1999). In the colon, the healing process is slower compared to the small intestine (see Brasken, 1991). Colonic anastomoses have prolonged presence of inflammatory reaction with polymorphonuclear leukocytes, and a slightly poorer vascularity during the first postoperative days. Also, mucosal repair is slower in the colon than in the small intestine. Similarly as in the skin wounds, healing intestinal anastomoses never gain the breaking strength of normal intestine. (see Graham et al, 1992; see Mast, 1997)

5.3. CHRONIC CUTANEOUS WOUNDS

When a wound fails to heal, it results in a chronic, nonhealing ulcer. The chronic ulcer usually develops medially above the malleoli as an irregular craggy area, and is often complicated by infections. Recurrency rate of a chronic leg ulcer is high. Leg ulcers are a common problem of the elderly, with e.g. 2% prevalence in Swedish population aged 50-89 years (Nelzen et al, 1996). The most common cause of a leg ulcer is chronic venous insufficiency (80-90%), followed by arterial disease and diabetic neuropathy (Baker et al, 1992). Other causes include vasculitis, malignancies, bacterial infections and pyoderma gangrenosum. Histologically, a venous ulcer is characterized by a discontinuous epithelium in the wound margin, the ulcer base being covered by fibrinous exudate with polymorphonuclear leukocytes. The underlying dermis contains numerous blood vessels surrounded by fibrin cuffs, and the remainder of the dermis consists of fibrous tissue with a variable number of inflammatory cells (Herrick et al, 1992). Extravasation of the red blood cells results in purpura as well as collections of hemosiderin and melanin. Prolonged presence of ECM molecules such as fibronectin, tenascin and chondroitin sulphate, and a greater number of B-cells and plasma cells is detected in chronic ulcers, compared to acute wounds (Loots et al, 1998). No differences in keratinocyte proliferation rates between normally healing and chronic wounds have been detected (Andriessen et al, 1995). Factors contributing to poor healing may be e.g. the following: 1) Keratinocytes may fail to migrate across the wound bed (see Falanga et al, 1994). 2) Fibrin cuffs surrounding the blood vessels may bind growth factors and proteins, and impede oxygenation and growth factor transportation to the wound area (Herrick et al, 1992; Highley et al, 1995). 3) Hypoxia may stimulate the fibrotic response and hence interfere with proper tissue repair. (see Ferguson and Leigh, 1998) 4) Excessive proteolysis may interfere with proper wound healing e.g. by degrading provisional matrices needed for cell migration or by inactivating growth factors (Grinnell et al, 1992). 5) Polymicrobial aerobic/anaerobic flora complicating all leg ulcers may delay the healing process (Dagher et al, 1978; Brook & Frazier, 1998). Thus, the pathogenesis of chronic wounds is multifactorial, and one of the reasons for impaired wound healing may be altered balance of MMPs and their inhibitors, TIMPs. (see Ferguson and Leigh, 1998; see Falanga et al, 1994)

5.4. INFLAMMATORY BOWEL DISEASE

Crohn´s disease and ulcerative colitis are chronic, inflammatory diseases of the gastrointestinal tract. They are sometimes associated with extraintestinal manifestations, e.g. uveitis, ankylosing spondylitis and skin lesions. Their origin is unknown, yet genetic predisposition, immunologic mechanisms, and environmental factors such as changes in the bacterial flora of the intestine and cigarette smoking, have been suspected as contributors to their pathogenesis. The global prevalence of these diseases is generally estimated to be greater than 1 per 500 inhabitants in Western countries, and most studies find ulcerative colitis to be more frequent than Crohn`s disease. (see Robbins et al, 1989b; see Fiocchi 1998; see Hugot et al, 1999).

Clinical manifestations of Crohn`s disease are variable. The beginning of the disease is characterized by attacks of fever, diarrhea and abdominal pain, which are spaced by asymptomatic periods. The inflammation in Crohn`s disease involves any level of the gastrointestinal tract, and leads to progressive damage. It presents with patchy, sharply demarcated inflammation of noncaseating granulomas and neutrophil abcesses affecting all layers of the intestine. Diseased muscularis mucosa and submucosa are characterized by lymphoid aggregates, increase in the number of smooth muscle cells, and presence of fragmented, disorganized collagen fibers. Deep-seated inflammation leads to deep ulcerations and complications typical of Crohn`s disease, namely fibrosis and fistula formation. (see Robbins et al, 1989b; see Podolsky, 1991; See Graham et al, 1992)

Ulcerative colitis presents as a relapsing disorder with attacks of bloody mucoid diarrhea followed by asymptomatic periods of varying lengths. The most feared complications of this disease are fulminant colitis and cancer. Ulcerative colitis affects the mucosal layers of the colon and rectum, invariably beginning in the rectum and gradually extending along the colon in a retrograde fashion. Histologically, it is characterized by continuous inflammation with an infiltrate of mononuclear cells, neutrophils, eosinophils and mast cells. Small mucosal hemorrhages may develop suppurative centers, crypt abcesses, which may give rise to small ulcerations, or rupture to underlying tissue. The inflammation in ulcerative colitis is more superficial than in Crohn`s disease, leads to ulcers that seldom extend beyond the submucosa, and to an increased turnover and depletion of the ECM. (see Robbins et al, 1989b; see Podolsky, 1991; See Graham et al, 1992)

5.5. MATRIX DEGRADING ENZYMES

Tissue remodeling is frequently encountered in physiological and pathological states such as wound healing, fetal development, joint inflammation, and cancer invasion and metastasis. Proteolytic activity of different cell types is essential for these conditions. The main groups of ECM-degrading proteinases are metalloproteinases, serine proteinases, and cysteine proteinases as divided upon their

active center. The serine proteinases, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), convert the zymogen plasminogen into plasmin, which can degrade several ECM components and activate proforms of certain matrix metalloproteinases. UPA is also able to degrade at least fibronectin, and activate gelatinase A (Keski-Oja et al, 1992). Cysteine proteinases cathepsin B, D, G, H, L and N are lysosomal in origin, but released into the ECM in some pathological conditions. The cysteine proteinase cathepsin G may contribute to ECM remodeling by activating latent collagenase-1. (See Saksela and Rifkin, 1988; Mignatti and Rifkin, 1993; Mauch et al, 1994)

5.5.1. Matrix metalloproteinases

Matrix metalloproteinases (matrixins, MMPs) are a family of structurally related, zinc-dependent endopeptidases. The first member of this family was found attacking triple-helical collagen in resorbing tadpole tails in metamorphosis (Gross & Lapiere, 1962). Today, at least 19 MMPs have been found (Table 2). They have been implicated in numerous physiological processes, e.g. reproduction, fetal development and wound healing (Stricklin et al, 1993; Stricklin et al, 1994a; Stricklin et al, 1994b; Rodgers et al, 1994; Inoue et al, 1995; Airola et al, 1998), and in pathological processes such as cancer cell invasion and metastasis, tissue degradation during inflammatory processes of various organs, lung diseases, multiple sclerosis, atherosclerosis and blistering skin diseases (see O`Connor & FitzGerald, 1994; see Cawston & Billington, 1996; see Jones & Walker, 1997; see Chandler et al, 1997; Knox et al, 1997; see Kähäri & Saarialho-Kere, 1997; Uitto et al, 1998). The family of the MMPs consists of collagenases, gelatinases, stromelysins, MT-MMPs, and others, as divided by their structure and function (Table 2). As a group these enzymes can *in vitro* degrade essentially all components of the ECM.

There are some structural features common to all MMPs. All members of the family share the propeptide domain that is lost upon activation, and the catalytic domain, which contains the zinc-binding site (see Woessner 1991, see Birkedal-Hansen et al, 1993). The hinge-region marks the transition to the COOH terminal domain. The hemopexin- or vitronectin-like COOH-terminal domain is likely to play a role in encoding substrate specificity and belongs to all MMPs except for matrilysin, which therefore is the smallest of the MMPs (see Birkedal-Hansen et al, 1993). In collagenase, for example, deletion of the C-terminal domain prevents it from cleaving native collagen (Murphy et al, 1992). The gelatinases possess an insert within the catalytic domain, that provides the enzymes with gelatin-binding properties (Goldberg et al, 1989). The transmembrane domain of the membrane-type MMPs localizes their action to the cell surface. The recently cloned MMP-23 exhibits sequence similarity with other MMPs, but displays a different domain structure, lacking a recognizable signal sequence and having short prodomain, and C-terminal domain with no hemopexin-like repeats (Velasco et al, 1999). (see Seiki, 1999)

5.5.1.1. Collagenases

Collagenases (collagenases-1, -2 and -3; MMPs-1, -8 and -13) are named after their unique capacity to degrade fibrillar collagen types I, II, III, V and IX. The fibrillar collagens are cleaved at a specific site to yield N-terminal ¾ and C-terminal ¼ fragments, which denature to gelatin at room temperature. Collagenase-1 degrades preferentially type III collagen, while collagenase-2 has

Table 2. Matrix metalloproteinases, their substrates, exogenous activators, and activating capacity

Enzyme	*Substrates*	*Activated by*	*Activator of*
Collagenases			
Collagenase-1 (MMP-1)	Collagen I, II, III (III>I), VII, VIII, X, gelatin, aggrecan, versican, proteoglycan link protein, L-selectin, entactin, tenascin, serpins, α2-macroglobulin, TNF precursor, MBP, IGFBP-3	MMP-3, -10, plasmin, kallikrein, chymase	MMP-2
Collagenase-2 (MMP-8)	Collagen I, II, III (I>III), VII, VIII, X, gelatin, aggrecan, fibronectin, laminin, serpins, α2-macroglobulin	MMP-3, -10, plasmin	ND
Collagenase-3 (MMP-13)	Collagen I, II, III (II>I or III), IV, IX, X, XIV, gelatin, aggrecan, perlecan, fibronectin, laminin, tenascin, fibrillin*, SPARC**, serpins, PAI	MMP-2,-3, -10,-14, -15, plasmin	MMP-2, -9
Gelatinases			
Gelatinase A, 72 kDa (MMP-2)	Gelatin, collagen I, IV, V, VII, X, XI, XIV, aggrecan, versican, proteoglycan link protein, fibronectin, laminin, laminin-5 (Giannelli et al, 1997), tenascin, fibrillin*, SPARC**, elastin, vitronectin, α2-macroglobulin, TNF precursor, MBP, IGFBP-3	MMP-1, -7, -13, -14, -15, -16, -24	MMP-9, -13
Gelatinase B, 92 kDa (MMP-9)	Gelatin, collagen IV, V, VII, X, XIV, aggrecan, versican, nidogen, proteoglycan link protein, fibronectin, fibrillin*, SPARC **, entactin, elastin, vitronectin, α1-antitrypsin, α2-macroglobulin, TNF precursor, MBP, angiostatin	MMP-2, -3, -13, plasmin	ND
Stromelysins			
Stromelysin-1 (MMP-3)	Collagen III, IV, V, IX, X, gelatin, versican, nidogen, aggrecan, perlecan, proteoglycan link protein, fibronectin, laminin, tenascin, fibrillin*, SPARC**, entactin, elastin, TNF precursor, MBP, IGFBP-3	Plasmin, kallikrein, chymase, tryptase, elastase, cathepsin G	MMP-1, -8, -9, -13
Stromelysin-2 (MMP-10)	Collagen III, IV, V, gelatin, nidogen, aggrecan, proteoglycan link protein, fibronectin, elastin	As stromelysin-1	MMP-1, -7, -8, -9,-13 (Nakamura et al, 1998)
Stromelysin-3 (MMP-11)	α1-proteinase inhibitor; weak activity on aggrecan, fibronectin, laminin	Furin	ND
Matrilysin (MMP-7)	Collagen IV, gelatin, versican (Halpert et al, 1996), nidogen, aggrecan, fibronectin, laminin, tenascin, SPARC**, elastin, vitronectin, MBP, angiostatin	MMP-3, plasmin	MMP-2
Metalloelastase (MMP-12)	Collagen IV, gelatin, nidogen, aggrecan, fibronectin, laminin, fibrillin*, elastin, vitronectin, α1-antitrypsin, TNF precursor, angiostatin	ND	ND
Membrane-type MMPs			
MT1-MMP (MMP-14)	Collagen I, II, III, gelatin, nidogen, aggrecan, perlecan, fibronectin, laminin, tenascin, vitronectin, fibrillin*	Plasmin, furin	MMP-2, -13
MT2-MMP (MMP-15)	Aggrecan, perlecan, fibronectin, laminin, nidogen, tenascin (d´Ortho 1997)	ND	MMP-2, -13
MT3-MMP (MMP-16)	gelatin, casein (Shofuda et al, 1997)	ND	MMP-2
MT4-MMP (MMP-17)	gelatin, TNF precursor (Wang et al, 1999)	ND	MMP-2
MT5-MMP (MMP-24)	ND (Pei 1999; Llano et al, 1999)	ND	MMP-2
Other MMPs			
MMP-18 & -19	gelatin (Sedlacek et al, 1998)	Trypsin	ND
Enamelysin (MMP-20)	Amelogenin (Llano et al, 1997)	ND	ND
MMP-21 & -22 & -23	ND (Gururajan et al, 1999; Velasco et al, 1999)	ND	ND

Modified from Kähäri & Saarialho-Kere, 1997; Chandler et al, 1997 and Imper & van Wart, 1998. *Ashworth et al, 1999.**Sasaki et al, 1997. ND not determined.

preference for monomeric type I and II collagens (Welgus et al, 1981; Hasty et al, 1987). Collagenase-3 is 10-fold more effective in degrading type II collagen, has a stronger gelatinolytic activity than collagenase-1, and a broader substrate specificity than the other collagenases (see Table 2) (Knäuper et al, 1996). *In vitro* collagenase-1 is expressed in various normal cell types such as keratinocytes, fibroblasts, endothelial cells, monocytes, macrophages, chondrocytes and osteoblasts (see Birkedal-Hansen et al, 1993). Unlike collagenase-1, collagenase-2 expression has only been detected in neutrophils, in chondrocytes in the presence of IL-1β, and in endothelial cells and synovial fibroblasts (Cole et al, 1996; Hanemaaijer et al, 1997). Collagenase-3 was originally cloned from breast cancer tissue (Freije et al, 1994) and its expression has been detected in developing bone, in osteoarthritic cartilage and rheumatoid synovial membrane, in periodontitis, and in malignancies such as melanoma and squamous cell carcinoma (Mitchell et al, 1996; Reboul et al, 1996; Johansson et al, 1997b; Johansson et al, 1997c; Uitto et al, 1998; Airola et al, 1999; Johansson et al, 1999).

5.5.1.2. Gelatinases

Gelatinases A and B (72 kDa and 92 kDa gelatinases; MMPs-2 and -9) cleave a number of peptide bonds in denatured collagen to yield small peptides. Other substrates for the gelatinases include types IV, V, VII and X collagen and elastin (see Kähäri and Saarialho-Kere, 1997). Gelatinase A has been identified in skin fibroblasts, keratinocytes, chondrocytes, endothelial cells, monocytes, osteoblasts and in a number of other normal and transformed cells (see Birkedal-Hansen et al, 1993). Gelatinase A knockout mice exhibit reduced angiogenesis, impaired primary tumor growth and decreased experimental metastases of B16-BL6 melanoma and Lewis lung cell carcinoma cells (Itoh et al, 1998). These mice suffer from a slight growing delay (Itoh et al, 1997). Gelatinase B is produced by keratinocytes, monocytes, alveolar macrophages, polymorphonuclear leukocytes and by numerous malignant cells but not by fibroblasts (see Birkedal-Hansen et al, 1993). Gelatinase B knockout mice are viable, but have delayed chondrocyte apoptosis, vascularization and ossification, resulting in abnormal lengthening of the skeletal growth plate (Vu et al, 1998). These mice do not develop blisters in an experimental model for bullous pemphigoid, despite the presence of neutrophils and antibodies to collagen type XVII (Liu et al, 1998), suggesting an important role for this enzyme in the pathobiology of pemphigoid.

5.5.1.3. Stromelysins

The stromelysin subgroup of MMPs includes stromelysins-1 (MMP-3) and -2 (MMP-10). Matrilysin (MMP-7) and matrix metalloelastase (MMP-12) can be included in this group. These enzymes have a broad substrate specificity, and they share the property of being able to activate other MMPs. Stromelysin-3 (MMP-11) is distantly related to other stromelysins. It was initially cloned from breast cancer tissue, and differs from other MMPs in that stromelysin-3 does not exhibit proteolytic activity towards ECM components (Basset et al, 1990; see Basset et al, 1993). The only known physiologic substrate for stromelysin-3 is α1-proteinase inhibitor (Pei et al, 1994).

Stromelysin-1 and stromelysin-2 are closely related structurally and have virtually identical substrate specificities. *In vitro* they are able to degrade e.g. gelatin, fibronectin, types IV and V collagens, elastin, and proteoglycan core proteins (Wilhelm et al, 1987; Murphy et al, 1991; see

Chandler et al, 1997). In addition, stromelysin-1 cleaves TNF-α precursor, α1-proteinase inhibitor and myelin basic protein (see Chandler at al, 1997). Stromelysin-1 is expressed by a diversity of cells and tissues, e.g. fibroblasts, keratinocytes, chondrocytes, endothelial cells and macrophages. Stromelysin-2 transcripts are expressed generally by normal or malignant cells of epithelial origin, at lower levels than stromelysin-1, and no expression has been detected in skin fibroblasts *in vivo*. Mice deficient in stromelysin-1 suffer from delayed healing of excisional wounds, due to impaired wound contraction (Bullard et al, 1999). However, keratinocyte migration and epithelialization proceed normally in these mice (Bullard et al, 1999). Stromelysin-1 deficiency does not affect collagen-induced arthritis in mice (Mudgett et al, 1998). (see Nagase, 1998)

Matrilysin, the smallest MMP due to the lack of hemopexin-domain, is often constitutively expressed in glandular epithelial cells. For example, epithelial cells of sweat glands produce matrilysin (Saarialho-Kere et al, 1995). It is also expressed by various cancer tissues and has been linked with tumor progression (see Wilson et al, 1998). Matrilysin is able to degrade proteoglycans, type IV and IX collagens, laminin, fibronectin, elastin, decorin, tenascin, globular domains of procollagens I and III and β4 integrin subunit (von Bredow et al, 1997; see Wilson et al, 1998). It is also able to activate procollagenase and process pro-TNFα, like stromelysin-1 (He et al, 1989, Gearing et al,1994). Matrilysin may regulate new blood vessel formation by cleaving plasminogen and generating angiostatin molecules (Patterson & Sang, 1997) and is likely to participate in the degradation of ECM in osteoarthritis (Ohta et al, 1998). Intestinal tumorigenesis is suppressed in mice lacking matrilysin (Wilson et al, 1997).

Human macrophage metalloelastase (HME) was initially found in alveolar macrophages of cigarette smokers (Shapiro et al, 1993). It is expressed in macrophages and the stromal cells of placenta (Belaaouaj et al, 1995) as well as in macrophage-like cells in breast cancer tissue (Heppner et al, 1996). Its substrates include elastin, type IV collagen, laminin, fibronectin, vitronectin and heparan and chondroitin sulphates, and it is able to process TNF-α precursor and generate angiostatin (Chandler et al, 1996; Gronski et al, 1997, Dong et al, 1997). HME participates in the degradation of elastic fibers at least in aneurysm formation, where it has been detected together with matrilysin, and in the pathogenesis of emphysema (Halpert et al, 1997; Hautamäki et al, 1997). Furthermore, HME is involved in the pathogenesis of skin diseases with elastic tissue abnormalities (Vaalamo et al, 1999; Saarialho-Kere et al, 1999). Mice deficient in HME undergo normal fetal and postnatal development without inflammatory stress (Hautamäki et al, 1997). These mice have impaired macrophage mediated proteolysis and matrix invasion (Shipley et al, 1996), increased numbers of macrophages in their lungs, and they do not develop emphysema in response to long-term exposure to cigarette smoke, unlike the wild-type mice (Hautamäki et al, 1997).

5.5.1.4. Membrane-type metalloproteinases

Membrane-type metalloproteinases (MT-MMPs-1, -2, -3, -4, and -5; MMPs-14, -15, -16, -17, and -24) are bound to the cell membrane, and take part in the activation of other MMPs besides their ECM-degrading activities (see Knäuper and Murphy, 1998). Maturation of the pro-form of at least MT1-MMP is regulated by an intracellular, furin-dependent pathway (Pei & Weiss, 1996; Sato et al, 1996). Extracellularly this enzyme can be activated by plasmin (Okumura et al, 1997). MT1-MMP was cloned as an activator of 72kDa progelatinase (Sato et al, 1994). It is expressed in lung, breast, colon, gastric, and head and neck cancers by stromal or cancer cells (Nomura et al, 1995; Okada et al, 1995; Polette et al, 1996). MT1-MMP is able to degrade fibrillar collagens type I, II and III,

gelatin, proteoglycan, fibronectin, vitronectin and laminin-1 (Ohuchi et al, 1997). Mice deficient in MT1-MMP suffer from ablation of collagenolytic activity, which leads to dwarfism, osteopenia, arthritis and connective tissue disease (Holmbeck et al, 1999). These mice are viable, but their mortality is increased. Like MT1-MMP, MT2-MMP, cloned from placenta cDNA library, is able to process progelatinase A into active form and shares also the substrate specificity with MT1-MMP (Takino et al, 1995; Pei et Weiss, 1996; d´Ortho et al, 1997). Expression of MT3- and MT4-MMPs has been found in various normal tissues by Northern analysis (Will et Hinzmann, 1995; Puente et al, 1996). These enzymes are able to process progelatinase A and hydrolyze gelatin, and MT3-MMP substrates include also casein, type III collagen, fibronectin, vitronectin, laminin-1, α1 proteinase inhibitor, and α2 macroglobulin (Shofuda et al, 1997; Sedlacek et al, 1998; Shimata et al, 1999). The recently cloned MT5-MMP is expressed at least during fetal development and in adult brain tissue in mice as well as in human brain, kidney, pancreas, and lung (Pei, 1999; Llano et al, 1999). MT5-MMP is able to activate progelatinase A, and is overexpressed in brain tumors thereby facilitating tumor progression (Pei, 1999; Llano et al, 1999).

5.5.1.5. Other matrix metalloproteinases

MMP-18 was cloned from human mammary gland cDNA. It is expressed in a wide variety of normal human tissues, and has closest identity with MMPs-1, -3, -10 and –11 (Cossins et al, 1996). MMP-19 was cloned from liver cDNA library, but it turned out to be identical to MMP-18 (Pendás et al, 1997). A novel collagenase, also named MMP-18 (*Xenopus* collagenase-4), was cloned from metamorphosing *Xenopus laevis* tadpoles (Stolow et al, 1996). Enamelysin (MMP-20) was cloned from odontoblasts, and is expressed in dental tissues, and degrades amelogenin (Llano et al, 1997). MMPs-21 and -22 genes were isolated and linked to the Cdc2L locus on human chromosome 1p36.3. Their catalytic domains are most closely related to stromelysin-3, and they express multiple mRNAs, some of which are derived in a tissue-specific manner by alternative spilicing (Gururajan et al, 1998). Essentially identical to MMPs-21 and –22 is MMP-23, which was cloned from an ovary cDNA library, and is expressed in testis, ovary, and prostate (Velasco et al, 1999).

5.5.2. Regulation of the matrix metalloproteinases

In normal tissues, the degradation and synthesis of ECM components is in balance. To maintain this steady state, there is a low basal level expression of certain MMPs, and the enzyme activity is tightly controlled. Inflammatory cytokines, hormones, growth factors, and cell-cell and cell-matrix interactions stimulate the expression of these enzymes through changes in transcription. Their activity is regulated by local activators, e.g. plasmin, and the specific inhibitors of the MMPs, TIMPs.

5.5.2.1. Regulation at the transcriptional level

Most cell types do not store MMPs, therefore the enzymes are expressed when needed, following certain signals. Generally the expression of MMPs can be stimulated by exogenous agents such as growth factors and cytokines. However, there are exceptions: e.g. gelatinase A is constitutively

expressed and responds poorly to common regulators, and within neutrophils, collagenase-2 is stored in granules, from where it is instantly released without preceding synthesis of the protein (see Birkedal-Hansen et al, 1993). Collagenase-2 expression by other cell types, such as chondrocytes, endothelial cells or synovial fibroblasts, is inducible (Cole et al, 1996; Hanemaaijer et al, 1997). IL-1, TNF-α, PDGF, and EGF stimulate expression of many MMPs via signals that depend, at least partially, on the activating protein-1 (AP-1) binding site. Regulatory elements of MMPs contain this site, except for gelatinase A and stromelysin-3. AP-1 site binds dimers of the Fos and Jun families and takes part in the basal and induced transcription of the MMP-promoter. AP-1 is necessary but not sufficient for transcriptional induction of MMPs in response to PMA (Auble & Brinckerhoff, 1991). Basal transcription as well as induction of MMPs rely on interaction and cooperation of AP-1 site with other cis-acting elements such as polyoma enhancer activator 3 (PEA3). Conserved PEA3 elements that bind members of the E26 transformation specific (ETS) family of transcription factors, are present in all but MMP-2 promoters. DNA binding and *trans*-activation capacity of both AP-1 and ETS transcription factors are regulated by phosphorylation by mitogen activated protein kinases (MAPKs). MAPKs are serine/threonine kinases that mediate signals from cell membrane receptors triggered by growth factors, cytokines, hormones, and cell-cell and cell-matrix interactions (see Westermarck & Kähäri, 1999). TGF-β, glucocorticoid hormones and retinoids are common downregulators of the transcription of the MMPs. However, regulation of MMP-expression is very cell type specific. TGF-β, for example, downregulates the expression of collagenase-1 and gelatinases in fibroblasts but upregulates the expression of these enzymes in keratinocytes (Edwards et al, 1987; Salo et al, 1991; Mauviel et al, 1996). (Woessner, 1991; see Benbow & Brinckerhoff, 1997).

In wound healing, cancer invasion and metastasis, and inflammation, the composition and organisation of the ECM changes constantly. Integrin receptors mediate information of these changes to cells through binding of different ECM component. The binding of certain ECM components may result in increased expression of MMPs. Antibodies to the fibronectin receptor that block adhesion of the fibroblasts to fibronectin, induce collagenase-1 and stromelysin-1 (Werb et al, 1989), and antibodies to β1 and β2 integrin subunits stimulate expression of gelatinase B, but not that of gelatinase A (Larjava et al, 1993b). Three-dimensional contact with collagen induces collagenase expression in fibroblasts (Mauch et al, 1989). This stimulation may be mediated by integrin receptors, since at least in osteogenic cell lines cultured within collagen gel, collagenase-1 expression is regulated by the collagen receptor α2β1 integrin (Riikonen et al, 1995). Not only changes in the ECM, but also in the cell itself may induce MMP expression; morphological changes in fibroblasts resulted in increased expression of collagenase-1 (Varedi et al, 1995). Increase in cell density downregulates EGF-mediated induction of collagenase and stromelysin (Colige et al, 1992), and mechanical injury resulted in increased expression of these enzymes in a cell line derived from vascular smooth muscle (James et al, 1993), which provide other examples of regulation of the MMPs by cell shape.

5.5.2.2. Zymogen activation

Most metalloproteinases are secreted as inactive precursor forms. The proteolytic activation of these zymogens takes place outside the cell, excluding furin-dependent intracellular activation of stromelysin-3 and MT1-MMP (Pei & Weiss, 1995; Pei & Weiss, 1996; Sato et al, 1996). The latency of MMPs is dependent on ”cysteine switch” formed by interaction of a conserved cysteine in

the propeptide with the zinc in the highly conserved catalytic site blocking the access of the substrate to the catalytic site (van Wart & Birkedal-Hansen, 1990). The catalytic site can be released by agents that are able to dissociate the covalent bond between the cysteine and the catalytic zinc. Organomercurials (APMA) and chaotropic agents can break the bond between cysteine and zinc. *In vivo* the activation most likely proceeds by proteolysis. Enzymes such as trypsin, plasmin and kallikrein process the proenzyme into active intermediate, which then autocatalytically cleaves itself to permanently active form. MMPs of the stromelysin subgroup are able to ”superactivate” other MMPs to fully active form. Activation of the MMPs by the membrane-bound MT-MMPs provide mechanisms for directing proteolytic activation to focal areas in the pericellular place. (see Woessner, 1991; see Murphy et al, 1999).

5.5.2.3. Inhibition of the activity of the metalloproteinases

The activity of the metalloproteinases may be inhibited by synthetic or natural inhibitors. The former include chelating agents such as EDTA and 1,10-phenanthroline, and inhibiting antibodies while the latter include specific inhibitors of MMPs, tissue inhibitors of metalloproteinases (TIMPs), and non-specific inhibitors, e.g. alpha 2-macroglobulin and alpha 1-antiprotease. Alpha 2-macroglobulin is abundant throughout the body, and may be important in controlling overall proteolytic activity. (see Birkedal-Hansen et al, 1993)

5.5.2.4. Tissue inhibitors of the metalloproteinases (TIMPs)

Tissue inhibitors of metalloproteinases are a family of secretory proteins that are able to inhibit matrix metalloproteinase activities through non-covalent binding of pre- or active forms of MMPs at molar equivalence. By inhibiting MMPs, TIMPs may influence also MMP-mediated processes such as processing of cytokines, degradation of growth factor binding proteins, and the release of ECM-bound growth factors (Gearing et al, 1995; Chandler, 1996; Whitelock et al, 1996; Martin et al; 1999). Up to date, a total of four TIMPs have been characterized, designated as TIMP-1, TIMP-2, TIMP-3 and TIMP-4. Members of this family have been detected in most human tissues and body fluids, and except for TIMP-3, they are present in a soluble form. (see Edwards et al, 1996; see Gomez et al,1997)

TIMP-1 is the oldest of the TIMPs. Like TIMP-2, it is able to inhibit all MMPs. However, TIMP-1 preferentially inhibits the activity of collagenase-1, while TIMP-2 is a stronger inhibitor of gelatinase A and B (Howard et al, 1991). TIMP-1 and TIMP-2 have been shown to inhibit tumour growth and metastasis in animal and cell culture studies (see Gomez et al, 1997). The expression of both TIMP-1 and –2 is induced in various cell lines and tissues. TIMP-2 forms a complex with MT1-MMP and takes part in the activation of gelatinase A in low concentrations, while high concentrations inhibit activation (Strongin et al, 1995; Butler et al, 1997).

Unlike other members of the TIMP-family, TIMP-3 is bound to the ECM (Leco et al, 1994), and has the capacity to inhibit TNF-α converting enzyme (TACE; ADAM-17) (Amour et al, 1998). It inhibits collagenase-1, gelatinases A and B, and stromelysin-1 as efficiently as TIMP-1 (Apte et al., 1995). By Northern analysis, it is expressed in breast carcinoma and in various normal adult tissues (Urìa et al, 1994; Apte et al, 1994). In situ hybridization studies have shown TIMP-3 expression

also during embryonic development, and in skin, mammary and colon cancers (Powe et al, 1997; Airola et al, 1998). In the eye, TIMP-3 forms a component of the Bruch`s membrane (Fariss et al, 1997).
The fourth tissue inhibitor of metalloproteinases, TIMP-4 (Greene et al, 1996; Leco et al, 1997), is a potent inhibitor of at least collagenase-1, gelatinases A and B, stromelysin-1 and matrilysin, but has preference for matrilysin, and for gelatinase A similarly as TIMP-2 (Liu et al, 1997). By Northern analysis, TIMP-4 is expressed in adult heart and in the kidney, placenta, colon, and testicles (Greene et al, 1996), and has been shown to inhibit the invasion potential of breast cancer cells *in vitro* and tumor growth and metastasis in nude mice *in vivo* (Wang et al, 1997).

The expression of TIMP-1 is upregulated by various growth factors and cytokines, e.g. TGF-β and IL-1β. The bifunctional effect of TNF is of interest; low concentrations of TNF stimulate TIMP-1 production while high concentrations suppress it (Ito et al, 1990). TIMP-2 expression is largely constitutive (DeClerk et al, 1994). In addition to regulation by cytokines, TIMP-3 expression is controlled in a cell-cycle dependent manner in certain cell types, and may serve as a marker for terminal differentiation (Wick et al, 1994).

Even though the best known physiological function for the TIMPs is their ability to regulate MMP-mediated proteolysis of the ECM, a growing number of other tasks have been established. For example, TIMP-1 was originally cloned on the basis of its erythroid-potentiating activity, and later TIMP-2 was found to share this property (Gasson et al, 1985; Docherty et al, 1985; Stetler-Stevenson et al, 1992). Both TIMP-1 and -2 are able to stimulate growth of various cell types, including keratinocytes and fibroblasts (Bertaux et al, 1991; Hayakawa et al, 1992; Nemeth & Goolsby, 1993; Hayakawa et al, 1994). Overexpression of TIMP-1 stimulates type IV collagen and laminin expression in rat mammary carcinoma cells, but has no effect on the expression of 72kDa gelatinase, stromelysin-1 or 92 kDa gelatinase (Yoshiji et al, 1998). Overexpression of TIMP-1 in rat vascular smooth muscle cells has no effect on cell proliferation, while that of TIMP-2 causes a dose-dependent reduction in proliferation (Baker et al, 1998). Overexpression of TIMP-3 induces DNA synthesis and promotes cell death by apoptosis (Ahonen et al, 1998; Baker et al, 1998). TIMP-3 induced cell death of at least human colon carcinoma cells is mediated by protection of the TNF-α-receptors from proteolytic cleavage (Smith et al, 1997). TIMP-1 and TIMP-3 are also able to inhibit angiogenesis by blocking endothelial cell response to angiogenic factors, and both TIMP-2 and TIMP-3 inhibit endothelial cell tube formation in vitro (Johnson et al, 1994; Anand-Apte et al, 1997).

5.6. MATRIX METALLOPROTEINASES IN CUTANEOUS WOUND REPAIR

Proteolytic degradation of the ECM is needed during re-epithelialization and stromal cell migration, neoangiogenesis and remodeling of the injured tissue. Because of their ECM degrading capacities, MMPs are most probably involved in distinct phases of wound healing. They may be needed in removal of devitalized tissue and remodeling of newly formed connective tissue, in keratinocyte migration, angiogenesis, and processing of certain growth factors (see Kähäri and Saarialho-Kere, 1997). Not only the contribution of MMPs, but also of other proteinases, e.g. serine proteinases, has been investigated in the context of wound healing. Plasminogen activation has been shown in iatrogenic human wounds, with subsequent removal of fibrin-containing provisional matrix (Schäfer

et al, 1994). The putative harmful effects of excess plasminogen activation on wound healing have been demonstrated by comparing wound fluids from chronic and normally healing wounds (Palolahti et al, 1993). A role for plasminogen activation in re-epithelialization has been suggested, since uPA, uPA receptor and type-1 plasminogen activator inhibitor (PAI-1) are expressed by keratinocytes during human and murine wound healing (Grøndal-Hansen et al, 1988; Rømer et al, 1991; Rømer et al, 1994). Furthermore, mice deficient in plasminogen suffer from severely impaired wound healing, with disturbed re-epithelialization and resolution of the fibrin clot (Rømer et al, 1996).

Collagenase-1 is the most investigated MMP in tissue repair. It is expressed by migrating keratinocytes in different types of ulcers: collagenase-1 mRNA and protein have been detected in pyogenic granulomas, venous and decubitus ulcers (Saarialho-Kere et al, 1992; Saarialho-Kere et al, 1993b). Wound edge keratinocytes in normally healing human wounds express collagenase-1 mRNA and protein from day 1 until complete re-epithelialization (Inoue et al, 1995). Human burn wound keratinocytes express collagenase-1 mRNA from day 2 (Stricklin et al, 1993), but no protein has been detected in the epithelium of burn wounds (Stricklin et al, 1994a). The activity of collagenase-1 reaches its maximum during the first days, and declines rapidly after re-epithelialization of acute porcine wounds (Ågren et al, 1992). Expression of collagenase-1 may be induced when disruption of the BM exposes the keratinocytes to the underlying matrix, that consists largely of fibrin and fibronectin (Clark et al, 1982). TGF-β and TNF-α are upregulated in wounds, and these growth factors stimulate expression of collagenase-1 at least in cultured keratinocytes, thus they might also contribute to collagenase-1 expression in wound margins (see Clark, 1995; Mauviel et al, 1996; Johansson et al, 1997a). The activity of collagenase-1 may be regulated by TIMP-1. In human burn wounds their expression has been reported to co-localize (Stricklin et al, 1993) but no TIMP-1 has been detected in basal keratinocytes in chronic ulcers (Saarialho-Kere et al, 1993b). Epidermal expression of collagenase-1 may be needed to facilitate keratinocyte migration by degrading fibrillar collagens.

Not only is collagenase-1 involved in re-epithelialization, but it is abundantly expressed by various types of stromal cells as well. Collagenase-1 mRNA is expressed in the granulation tissue of human and porcine burn wounds (Stricklin et al, 1993; Stricklin et al, 1994b). Histologically defined, the cells responsible for collagenase-1 expression may be macrophages, fibroblasts and endothelial cells. Due to its ability to cleave fibrillar collagens, collagenase-1 undoubtedly participates in the remodeling of the collagenous matrix. The regulation of collagenase-1 in the dermis is likely to differ from that in the keratinocytes. E.g TGF-β upregulates collagenase-1 expression in cultured keratinocytes, but downregulates its expression in fibroblasts (Mauviel et al, 1996). The upregulation of fibroblast collagenase-1 may be induced by EGF or bFGF, as these growth factors stimulate collagenase-1 expression at least in cultured quiescent human MRC-5 fibroblasts (Edwards et al, 1987). According to an *in vitro* study with rabbit synovial fibroblasts, fibronectin fragments bound to fibronectin receptor induce collagenase-1 expression (Werb et al, 1989). Thus, degraded fibronectin in the wound bed may result in fibroblast collagenase expression. However, granulation tissue fibroblasts are a cell population distinct from MRC-5/synovial fibroblasts, and their responses to specific stimuli may vary from each other.

The gelatinases are differentially expressed and regulated during wound healing. Gelatinase A is expressed by fibroblasts by both resting and healing human oral mucosa and skin (Oikarinen et al, 1993; Salo et al, 1994). No induction has been detected in the connective tissue cells of the wound margin or the epithelium of wounded oral mucosa or skin (Saarialho-Kere et al, 1993b; Salo et al, 1994), unlike in porcine burn wounds, where gelatinase A mRNA is expressed mainly in the

remodeling dermis adjacent to regenerating epidermis (Stricklin et al, 1994b). The activity of gelatinase A during porcine skin wound healing remains rather stable, yet higher than in normal skin, suggesting a role in prolonged remodeling of the matrix (Ågren, 1994). In human burn wound fluid, gelatinase A appears at day 3-4 and the levels are low compared to gelatinase B (Young & Grinnell, 1994). Cytokines such as TGF-β, bFGF, TNF-α or IFN-γ do not induce gelatinase A expression in cultured wound fibroblasts (Salo et al, 1994).

Strong expression for gelatinase B mRNA is detected in basal and suprabasal keratinocytes in nonwounded oral mucosa (Salo et al, 1994) which is in discrepancy with previous findings of the absence of gelatinase B mRNA in normal skin (Pyke et al, 1992). Only the basal, migrating keratinocytes express gelatinase B mRNA after oral mucosal incision, and cultured keratinocytes enhance MMP-9 production after stimulation with TGF-β, TNF-α or IL-1β (Salo et al, 1994). In regenerating human skin, gelatinase B mRNA is strongly expressed by basal keratinocytes in suction blisters (Oikarinen et al, 1993), and may be regulated by extracellular factors via integrin receptors (Larjava et al, 1993b). Gelatinase B mRNA expression is induced in the granulation tissue as well (Salo et al, 1994). Analysis of gelatinase activity after burn injury and in skin wounds reveals that gelatinase B is rapidly induced after injury, reaches maximum activity at 4-5 days after injury, and gradually declines (Young & Grinnell, 1994; Ågren et al, 1994).

Thus, the gelatinases appear to have distinct roles during wound healing. Gelatinase A participates in the long-term remodeling of the dermis, while gelatinase B is involved in reepithelialization. Chronic wound fluids contain elevated levels of gelatinases, which indicates that excess of these enzymes may disturb wound healing (Wysocki et al, 1993; Bullen et al, 1995). High levels of gelatinase B may, at least partly, originate from polymorphonuclear leukocytes of secondarily infected chronic ulcers (Herrick et al, 1992; see Birkedal-Hansen et al, 1993; Brook & Frazier, 1998).

In vitro stromelysins-1 and -2 are able to degrade important components of the dermal matrix and basement membranes, e.g. type IV collagen, elastin, laminin, and fibronectin (see Nagase, 1998). The wide variety of substrates is reflected in the pattern of expression of stromelysin-1 in chronic wounds. It is expressed by basal keratinocytes adjacent to but distinct from the migrating front, and abundantly by stromal cells (Saarialho-Kere et al, 1994). Unlike stromelysin-1, stromelysin-2 is expressed in chronic ulcers by the migrating front of keratinocytes and is not expressed by the cells of the wound matrix (Saarialho-Kere et al, 1994). Not only are these two enzymes expressed by distinct cell populations in chronic wounds, but also their regulation is indivudual. Keratinocytes in the migrating front may be induced to express stromelysin-2 by EGF, TGF-α, or TNF-α, as these cytokines induce stromelysin-2 in keratinocyte cultures, and are released in the wound area by platelets and macrophages (Windsor et al, 1993; see Clark, 1995). However, these agents are unable to induce stromelysin-1 in keratinocytes, which suggests a different stimulus for this enzyme (Windsor et al, 1993). Thus, stromelysin-2 appears critical to keratinocyte migration, whereas stromelysin-1 may be involved in the remodeling of the newly formed BM and of the wound matrix. Furthermore, due to their ability to activate procollagenase (Suzuki et al, 1990), they may contribute to collagenase-mediated proteolysis. Stromelysin-3 expression has been shown in late stages of cutaneous wound healing in areas of inflammatory fibrosis (Wolf et al, 1992).

5.7. MATRIX METALLOPROTEINASES IN INTESTINAL DISEASE

MMPs in intestinal inflammation and wound healing are a rather uninvestigated area compared to the vast amount of literature on repair processes of the skin. An immunohistochemical study by Bailey et al (1994) was performed to investigate the role of collagenase-1, gelatinases A and B, and stromelysin-1 in the intestinal connective tissue changes in Crohn`s disease and colitis ulcerosa. The expression of collagenase-1 was observed in few isolated mononuclear cells, and that of gelatinase A in occasional polymorphonuclear leukocytes, in both normal and IBD samples. In Crohn`s disease, gelatinase B protein was expressed by polymorphonuclear leukocytes throughout the intestinal wall. Stromelysin-1 protein was expressed by few mononuclear cells in normal tissue, with a slight increase in diseased samples. According to this study, IBD associated changes in the intestinal wall may partly result from MMP activity.

Most studies on MMPs in the intestine have focused on malignancies. Matrilysin expression, but not stromelysin-1 or –2, has been shown in human gastric and colon carcinomas, suggesting that inappropriate expression of matrilysin may contribute to the neoplastic phenotype (McDonnel et al, 1991). No expression of this enzyme was detected in normal tissue or other than carcinoma cells (McDonnel et al, 1991). Another study provides evidence that matrilysin is expressed also by premalignant cells of colon adenomas, but the levels of enzyme activity are lower than in carcinomas (Yamamoto et al, 1994). Furthermore, truncated fibronectin induces matrilysin expression in cultured WiDr cells (Yamamoto et al, 1994). No expression was detected in samples of mildly inflamed regions of ulcerative colitis or normal intestine (Yamamoto et al, 1994). A role for matrilysin in early events of tumorigenesis, and stromelysins-1 and –3 and gelatinase A in later stages of tumor progression is suggested in a study by Newell et al (1994). According to them, expression of matrilysin mRNA localizes mainly to adenoma/carcinoma cells, and of the other MMPs to stromal cells of carcinoma tissue, while TIMP-1 mRNA is prominent in both carcinoma and stromal cells. The relevance of matrilysin in intestinal tumors is further substantiated by the suppression of intestinal tumorigenesis in mice lacking the metalloproteinase matrilysin (Wilson et al, 1997). Gelatinase A and its inhibitor TIMP-2 mRNAs are expressed in colorectal adenomas and carcinomas, mostly by peritumoral fibroblast-like cells (Poulsom et al, 1992). However gelatinase A protein localizes mainly to neoplastic epithelial cells.

Not only MMPs, but also their activator uPA, are involved in intestinal neoplasias. According to immunohistochemical studies, tumor infiltrates contain more uPA positive cells than normal tissues (Kohga et al, 1985; Grøndahl-Hansen et al, 1991). UPA is produced by merely fibroblast-like/endothelial cells of the stroma (Grøndahl-Hansen et al, 1991) or, according to another study, also by tumor epithelial cells (Kohga et al, 1985).

5.8. MATRIX METALLOPROTEINASES IN WOUND REPAIR IN OTHER TISSUES

Matrix metalloproteinases are involved in wound healing processes in tissues such as cornea and respiratory epithelia. Matrilysin and gelatinases A and B mRNAs and proteins, and stromelysin-1, TIMP-1 and TIMP–2 proteins are upregulated during rat corneal wound healing after an excimer

laser keratectomy (Ye & Azar, 1998; Chung-Shien Lu et al, 1999). The overexpression of MMPs in corneal wounds may delay wound healing, as has been shown in thermally injured rat and rabbit corneas (Fini et al, 1996). *In vitro* studies on experimental wounds in human surface respiratory epithelial cell cultures or bronchial mucosa organ culture have shown increased expression of stromelysins-1 and –3 in migratory cells (Buisson et al, 1996). An upregulation of matrilysin after injury to airway epithelium suggests a role for it in facilitating re-epithelialization (Dunsmore et al, 1998). The role of MMPs in alveolar epithelial wound repair is further substantiated by the finding that exogenous collagenases enhance migration of mechanically injured alveolar epithelial cell culture (Planus et al, 1999).

5.9. MATRIX METALLOPROTEINASES AS TARGETS OF WOUND THERAPIES

Bacterial collagenase has been used for many years in debriding necrotic ulcers (Mekkes et al, 1997). This enzyme is under physiological conditions more effective in solubilizing both native and denatured collagens than endogenous collagenases, thus severing the undenatured collagen fibers holding the necrotic tissue within the wound bed (see Baharestani, 1999). A potent activator of wound repair, glycyl-l-histidyl-l-lysine-Cu^{2+}, enhances expression of the gelatinases as well as ECM deposition (Siméon et al, 1999), which argues against the suggested harmful effect of excess MMPs during wound healing. In the future, synthetic selective inhibitors of the MMPs may offer new treatment options for those suffering from chronic ulcers. They are small peptide analogues of fibrillar collagens, which interact with the zinc in the catalytic site of MMPs and inhibit their activity (see Kähäri & Saarialho-Kere, 1997; see Kähäri & Saarialho-Kere, 1999). Indeed, the synthetic collagenase inhibitor GM6001, has been studied on murine wounds, and was found to diminish the inflammatory response and angiogenesis, and to increase incisional wound strength (Witte et al, 1998). However, caution is required when using broad spectrum MMP-inhibitors in wound healing trials, since a growing number of studies indicate that certain MMPs serve beneficial functions in wound repair. Also tetracyclines, retinoids and glucocorticoids may influence wound healing through MMP-mediated cascades (see Kähäri & Saarialho-Kere, 1997; see Kähäri & Saarialho-Kere, 1999).

6. AIMS OF THE STUDY

Matrix degradation and cell migration are intrinsic components of wound healing. Matrix metalloproteinases comprise an important group of matrix-degrading enzymes, which are involved in these processes during wound repair. Since it has been assumed, that excessive proteolysis may delay wound healing, we planned to study the expression profiles of MMPs and their inhibitors in normally healing and chronic wounds.

The specific aims of the study were as follows:

1. To study the spatial and temporal expression patterns of various MMP:s (collagenases-1 and -3, stromelysins-1 and -2, matrilysin and metalloelastase) in normally healing cutaneous wounds vs. chronic ulcers.

2. To define the expression of TIMPs-1, -2, -3 and -4 during cutaneous wound healing *in vivo*.

3. To investigate the expression of MMPs (collagenases-1 and -3, stromelysins-1 and -2, matrilysin and metalloelastase) and their inhibitors (TIMPs-1 and –3) in chronic intestinal erosions/ulcerations caused by inflammatory bowel disease.

7. MATERIALS AND METHODS

7.1. TISSUE SAMPLES

The study was approved by the Ethics Committee of the Department of Dermatology, Helsinki University Central Hospital. All tissue samples were formalin-fixed and paraffin embedded. Informed consent was obtained from individual subjects for all procedures.

Chronic dermal wounds. Human skin samples (n=14) were collected from patients with chronic leg ulcers undergoing excision and skin grafting prodecures at the Department of Plastic Surgery, Helsinki University Central Hospital. The patients were aged between 50 to 91 years. The ulcers were 3 months to 10 years old and had not responded to conservative treatment. Archival specimens of chronic ulcers (n=6) / vasculitis (n=5) were obtained from the Department of Pathology, Helsinki University Central Hospital.

Normally healing human wounds. As controls for acute full thickness wounds (n = 25) biopsies of normally healing donor areas on the anterior thigh were obtained from patients of the Department of Dermatology, University of Helsinki, undergoing pinch grafting procedure (Ceilley et al, 1977). On day 0, several pieces of skin measuring 8 mm in diameter and including a portion of the dermis centrally, were excised from the donor area and transferred to the ulcer area. Biopsies containing the donor area wound were taken 1, 2, 3, 4, 5, 6, 7 and 9 days postwounding from patients aged between 55 to 94 years.

Suction blisters were obtained from Central Military Hospital, Helsinki. Blisters were induced on the abdominal skin of four healthy volunteers under 30 years of age using a Dermavac device as described previously (Kiistala & Mustakallio, 1968). Biopsies were taken 2, 4, and 9 days after induction of the blister.

Normally healing experimental pig wounds. 3-cm long full-thickness incisional wounds were made, using a surgical steel scalpel, on the skin of an anesthesized piglet. The wounds were closed with Prolene sutures and, therefore, healed by primary intent. To ensure adequate sampling, three wounds were excised at each time point: 1, 3, 5, 7, 10 and 14 days after wounding. The samples were fixed in 10% formaldehyde, bisected in a plane perpendicular to the long axis of the wound, and embedded in paraffin.

Chronic human gastrointestinal ulcers. Specimens of duodenal ulcer (n=3), gastric ulcer (n=5), ulcerative colitis (n=12), Crohn`s disease (ileum, n=7; colon, n=8), ischemic colitis (n=8), histologically normal colon (n=3) and ileum (n=4) were obtained from the Department of Pathology, University of Helsinki. All disease samples represented active, ulcerative phase of the disease and were from adult patients.

Experimental model for intestinal anastomoses. Animal studies were approved by the Regional Committee for Ethics in Animal Research and Administrative Board at Helsinki University Central Hospital. Adult male Wistar Rats (300g) were anesthesthetized with a single intramuscular injection of ketamine (40mg/kg Vetalar®). The jejunum was cut at laparotomy with a steel scalpel

approximately 10 cm from the Ligamentum of Treitz. Jejunal anastomoses were performed with one layer of interrupted 6-0 polypropylene sutures (Prolene®, Ethicon, Norderstedt, Germany). After the surgery the animals had free access to food and water. Animals were sacrificed at 1, 3, 4, 7, 9 and 14 days by an overdose of pentobarbital. To ensure adequate sampling, anastomoses were obtained from two separate animals for each time point. Tissues were embedded in paraffin and cut at 5 μm.

7.2. RNA PROBES

The production and specificity of the anti-sense human collagenase-1, stromelysin-1, stromelysin-2, matrilysin, uPA, TIMP-1 and TIMP-3 (McDonnel et al, 1991; Busiek et al, 1992; Saarialho-Kere et al, 1992; Sudbeck et al, 1992; Saarialho-Kere et al, 1994; Airola et al, 1995; Airola et al, 1998) probes have been described. The MMP-13 cDNA plasmid MMP13HT3 (Johansson et al, 1997a) was linearized within the multiple cloning site with HindIII and EcoR1 to allow transcription of anti-sense (corresponding to nucleotides 1532-2042) and sense RNAs, respectively. The HME cDNA used as a template was a kind gift from Steven Shapiro (Pulmonary Department, Washington University, St Louis). The 650 bp fragment (600-1250) was generated by PCR using the primers CAT ACG ATT TAG GTG ACA CTA TAC and TAA TAC GAC TCA CTA TA, resulting in a product with SP 6 RNA polymerase recognition element at the 3´end and a T7 element at the 5´end. Both antisense and sense probes were transcribed from this polymerase chain reaction product. The specificity of the probes was confirmed by sequencing. The murine stromelysin-2 cDNA used as a was kindly provided by Sabine Werner (Max-Planck-Institut für Biochemie, Martinsried, Germany) (Madlener et al, 1996). As a control for nonspecific hybridization, sections in each experiment were hybridized with ^{35}S-labelled sense RNA from a bovine tropoelastin cDNA. The validity of this probe as a negative control has been confirmed by Northern and by *in situ* hybridization assays (Prosser et al, 1989; Saarialho-Kere et al, 1992; Saarialho-Kere et al, 1993b). In addition, negative control probes transcribed from collagenases-1 and -3, stromelysins-1, matrilysin, HME, uPA as well as TIMP-1 and -3 cDNAs in sense orientation were used.

Table 3. Probes and their sources

Probe (Study)	*Transcribed from bases/ Genbank accession number*	*Source of the template*
Collagenase-1 (I, II, III)	1-550 (550 bp)/ M13509	Dr Gregory Goldberg
Collagenase-3 (III, IV)	1532-2042 (511 bp)/NM_002427	Dr Carlos López-Otín
Stromelysin-1 (I, II, III)	1584-1801 (217 bp)/J03209	Dr Markku Kurkinen
Stromelysin-2 (II, IV)	1568-1743 (176 bp)/X07820	Dr Henning Birkedal-Hansen
Matrilysin (I, II)	14-813 (800 bp) /Z11887	Dr Lynn Matrisian
Macrophage metalloelastase (IV)	600-1250 (651 bp)/L23808	Dr Steven Shapiro
TIMP-1 (I, II, V)	1-313 (313 bp)/X03124	Dr David Carmichael
TIMP-3 (IV, V)	282-917 (636 bp)/U14394	Dr Veli-Matti Kähäri
UPA (II)	835-1661 (827 bp)/K03226	Dr Antti Vaheri
Stromelysin-2, murine (IV)	8-219 (212 bp)/X64020	Dr Sabine Werner

TIMP, tissue inhibitor of metalloproteinases; UPA, urokinase plasminogen activator

The cDNA probes for stromelysin-2 and HME were amplified by polymerase chain reaction (PCR). The template and primer concentrations were optimized for each probe. First, the reagents (primers, template, nucleotides, reaction buffer, and the DNA polymerase) were mixed on ice. The PCR was started with initial denaturing in 94°C for 2,5 minutes. This was followed by 35 cycles consisting of 1. 30 sec of denaturing (94°C) 2. 45 sec of annealing (55°C) and 3. 45 sec of elongation (72°C). The PCR-product was purified by chloroform-isoamylalcohol extraction and ethanol precipitation. The correct size of the PCR-product was checked by running it into an ethidium bromide-stained agarose gel, and by visualizing it under UV-light.

7.3. IN SITU HYBRIDIZATION

In situ hybridization was performed on 5-µm sections as described in detail (Prosser et al, 1989). All samples were treated with proteinase K and were washed in 0.1 M triethanolamine buffer containing 0.25% acetic anhydride. Sections were covered with 50 µl of hybridization buffer containing 2.5-5 x 10^4 cpm/µl of ^{35}S-labelled anti-sense or sense RNA probe. After hybridization at 50°C to 55°C for 18 hours in a humidified chamber, the slides were washed under stringent conditions, including treatment with RNAse A to remove unhybridized probe. Following 10 to 45 days of autoradiography, the photographic emulsion was developed, and slides were stained with hematoxylin and eosin. Samples previously positive for collagenase-1, stromelysin-1 and stromelysin-2 (cutaneous wounds), for matrilysin (normal skin), for collagenase-3, macrophage metalloelastase, TIMP-1 and -3 (breast and colon carcinomas) and for uPa (cutaneous wounds) were used as positive controls in each experiment.

7.4. IMMUNOHISTOCHEMISTRY

Immunohistochemistry was performed on sections serial to those used for *in situ* hybridization. The peroxidase-antiperoxidase technique was applied using Vectastain Elite ABC kit (Vector Laboratories, Inc., Burlingame, CA). After deparaffinazation and dehydration, the endogenous proxidase activity was blocked with 0.3%-0.6% hydrogen peroxide. Non-specific staining was blocked by treatment with normal serum. The monoclonal primary antibodies were incubated for one hour at 37°C, and the polyclonal primary antibodies overnight at 4°C in a humidified chamber. After incubation with the primary antibody (see Table 3 for the *antibodies* used), the secondary biotinylated antibody was added, followed by avidin-biotin-peroxidase complex. Diaminobenzidine or aminoethylcarbazole (Ki-67, CD3, CD31) were used as chromogenic substrates and Harris hematoxylin as counterstain, as described in detail (Saarialho-Kere et al, 1993b). If necessary, sections were pre-treated with 10 mg/ml trypsin, 1 mg/ml protease type XXVII (Sigma Chelmical Co., St. Louis, MO), or antigen retrieval as described (von Boguslawski, 1994). Controls were performed with mouse preimmune ascites fluid or with rabbit pre-immune serum.

Combined immunohistochemistry and *in situ* hybridization was performed as described in detail (Ranki et al, 1995). After RNAse-free immunohistochemical staining with CD68 antibody, the samples were treated with 4% paraformaldehyde, washed with diethylpyrocarbonate-treated phosphate-buffered saline, and hybridized for collagenase mRNA with ^{35}S-labeled RNA as described (Prosser et al, 1989). After autoradiography, the slides were developed and stained with Mayer hematoxylin.

Table 4. Antibodies and their sources.

Antibody	*Source*	*Study*
anti-gelatinase A	IM33L, Calbiochem	V
anti-stromelysin-1	prof. Howard Welgus, Washington University, USA	II
anti-matrilysin	prof. Howard Welgus, Washington University, USA	I, IV
anti-TIMP-2	TIMP-2, Fuji Chemicals	V
anti-TIMP-3	IM43L, Calbiochem,	V
anti-TIMP-4	TIMP-4, Triple Point Biologics	V
anti-CD3	A452, Dako	IV
anti-CD20	U7021, Dako	IV
anti-CD31	M823, Dako	IV
anti-CD68	M814, Dako	I, III, IV
anti-Ki-67	0505, Immunotech	I, III. V
anti-type I procollagen	MAB1912, Chemicon	III, IV
anti-laminin-1	L-8217, Sigma Chemical Co	I
anti-laminin-5	prof. Karl Tryggvason, Karolinska Institut, Stockholm, Sweden	IV
anti-type IV collagen	M785, Dako	IV
anti-fibronectin	A245, Dako	I
anti-smooth muscle actin	6582, Bio-Makor	IV

7.5. CELL CULTURES

Normal human skin fibroblasts from a 26-year-old healthy volunteer were maintained in Dulbecco`s modification of Eagle`s medium (DMEM, Flow Laboratories, Irvine, Scotland, UK), 2 mM glutamine, 100 IU penicillin-G per ml, and 100 µl streptomycin per ml. Collagen gels were prepared with bovine dermal collagen, Cellon (Strassen, France), containing 95% type I collagen and 5% type III collagen. Eight volumes of Cellon were mixed with one volume of 10× concentrated DMEM and 1 volume of NaOH (0.05 M) in 0.2 M N-(2-hydroxyethyl)piperazine-N`-(2-ethanesulfonic acid) and kept on ice. Cells were trypsinised, resuspended in DMEM supplemented with 10% fetal bovine serum, mixed into a neutralized Cellon solution, and transferred into six-well plates. The plates were incubated at 37°C for 1 h for collagen polymerization. After this, DMEM supplemented with 10% fetal bovine serum was added and the gels were detached from the sides of the wells. The gels were incubated for 48 h before releasing the cells from the gels. As a control, fibroblasts were plated as monolayer and cultured in DMEM supplemented with 10 % fetal bovine serum for 48 h. Promonocytic U937 cells and CRL-1995 fibroblasts were cultured as previously described (Wilhelm et al, 1997; Saarialho-Kere et al, 1993c). Human alveolar macrophages were isolated from healthy adult smokers by saline bronchoalveolar lavage and cultured as described (Campbell et al, 1991).

7.6. NORTHERN BLOT ANALYSIS

To isolate total RNA from the dermal fibroblasts inside collagen gels, the gels were briefly treated with 0.5 mg collagenase (type II, Sigma) per ml in phosphate-buffered saline (pH 7.4) with 1 mM $CaCl_2$. Total cellular RNA was isolated from all cell types studied by using the single-step method (Chomczynski & Sacchi, 1987). Aliquots of total RNA (10-17 μg) were fractionated on 0.8% agarose gel containing 2.2M formaldehyde, transferred to a Zeta-Probe filter (Bio-Rad, Richmond, CA) by vacuum transfer (VacuGene XL; LKB, Bromma, Sweden), and immobilized by heating at 80°C for 30 min. The filter was prehybridized for 2 h and subsequently hybridized for 20 h with ^{32}P labeled cDNAs (Thomas, 1980) for human collagenase-1 (Goldberg et al, 1986) and human collagenase-3 (Johansson et al, 1997a). The [^{32}P]cDNA-mRNA hybrids were visualized with autoradiography.

8. RESULTS

8.1. CUTANEOUS WOUNDS (II, III, V)

Table 5. Typical patterns of MMP- and TIMP mRNA expression in normally healing cutaneous wounds and chronic ulcers

	Normally healing wounds						*Chronic ulcers*	
	1-2 days old		3-5 days old		6-11 days old			
MMPs	e	s	e	s	e	s	e	s
Collagenase-1	+	+	+	+	-	+	+	+
Collagenase-3	-	-	-	-	-	-	-	+
Stromelysin-1	+	+	+	+	-	+	+	+
Stromelysin-2	-	-	+	-	-	-	+	-
Matrilysin	-	-*	-	-*	-	-*	-	-*
Metalloelastase	-	-	-	-	-	-	-	+
TIMPs	e	s	e	s	e	s	e	s
TIMP-1	-	+	+	+	-	+	-	+
TIMP-3	-	+	+	+	-	+	-	+

*Matrilysin expression was detected in sweat glands in all types of cutaneous wounds.
MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; e, epidermal mRNA expression; s, stromal mRNA expression

Collagenase-1 mRNA expression was detected in the basal and suprabasal keratinocytes bordering the ulcer in both normally healing wounds, beginning the first postoperative day, and chronic wounds. The signal was the strongest in the keratinocytes closest to the wound edge. In chronic wounds, the number of positive keratinocytes was generally greater than in normally healing wounds, in which the expression was shut off after completion of re-epithelialization. Cells of the granulation tissue showed a strong expression for collagenase-1 mRNA in both normally healing and chronic wounds. Histologically, the cells were either plump, macrophage/activated fibroblast-like or spindle, fibroblast-like. Since it is difficult to define the cell type purely histologically, immunostaining for *procollagen-1*, which is found inside of activated fibroblasts, was performed (McDonald et al, 1986). According to this staining, many of the positive cells were activated fibroblasts. (II,III)

Collagenase-3 mRNA was not expressed in the epithelium of any dermal wounds nor in the stroma of normally healing wounds. An abundant expression of collagenase-3 was found in plump, macrophage-like/activated fibroblast-like as well as spindle, fibroblast-like cells in the chronic wound stroma of all samples investigated. The signal for collagenase-3 was generally located deeper in the stroma than the signal for collagenase-1. The areas with collagenase-3 positive cells exhibited either capillary proliferation or marked fibrosis. To define the cell type responsible for collagenase-3 expression, immunohistochemical stainings for activated fibroblasts (*procollagen-1*), macrophages (*CD-68*) (Pulford et al, 1989), and myofibroblasts (*α-smooth muscle actin*), which are numerous in wound stroma, were performed. Colocalization of *procollagen-I* positivity and

collagenase-3 mRNA showed that at least part of these cells were fibroblasts. Stainings for *CD-68* and *α-smooth muscle actin* revealed that the positive cells were not macrophages nor myofibroblasts. (III)

Stromelysin-1 mRNA was expressed in many of the chronic as well as re-epithelializing normally healing wound samples, in basal keratinocytes a short distance from the migrating tip. In agreement with the *in situ* data, immunohistochemical staining for *stromelysin-1 protein* was detected in basal keratinocytes only in 2 days old normally healing wounds. (II)
Since stromelysin-1 can activate collagenase-3 by a two-step mechanism (Knäuper et al, 1996), a subset of sections serial to those positive for collagenase-3 mRNA were hybridized with a cDNA probe for this enzyme. Some chronic wound samples had stromal signal for stromelysin-1 (II,III), but in areas that were negative for collagenase-3 mRNA (III). Like chronic ulcers, a subset of acute wound samples had stromelysin-1 positive cells in the stroma. (II)

Stromelysin-2 mRNA was detected in 3- and 5-day-old normally healing wounds and in a majority of chronic wound samples in the migrating front of the keratinocytes. No expression was detected in samples with intact epidermis, nor in the stroma. (II)

Matrilysin mRNA was only detected occasionally in the ductal cells of the sweat glands, with no epithelial or stromal expression in any wound samples investigated, which is in agreement with previous findings on matrilysin in skin (Saarialho-Kere et al, 1995). (II)

Urokinase plasminogen activator is a potent activator of MMPs (see Birkedal-Hansen et al, 1993). To assess whether it would be involved in the activation of MMPs expressed by the migrating keratinocytes, its expression was studied in normally healing and chronic wounds. We found expression for *uPA mRNA* in a majority of chronic ulcer samples in wound edge keratinocytes, and in a similar location in normally healing wounds until day 5, when the expression was shut off after re-epithelialization. Also, stromal signal for uPA mRNA was detected in many samples of both normally healing and chronic wounds. (II)

TIMP-1 mRNA was never detected in the epidermis of chronic wounds, while in some normally healing wound samples, the basal keratinocytes next to the migrating front showed signal for TIMP-1 until re-epithelialization. Stromal signal for TIMP-1 was detected in almost all samples investigated. In acute wounds, the signal was most prominent in the vicinity of blood vessels, while chronic ulcers showed a more widespread pattern of distribution of stromal TIMP-1 mRNA. (II, V)

TIMP-2 protein was detected around the migrating epithelial tip as well as in the tissue below the eschar in both normally healing wounds and suction blisters. In chronic wounds, TIMP-2 was abundantly expressed by spindle, fibroblast-like stromal cells, but in only few samples in the necrosis covering the epithelial tip. TIMP-2 protein was also found between the epithelial cells of stratum granulosum and upper layers of stratum spinosum in all sample types. Secreted *72 kDa gelatinase* protein was found in great amounts near the epithelial edge, and in TIMP-2 positive stromal areas in chronic ulcers. (V)

TIMP-3 mRNA was expressed in the epithelium of 3- to 5-day-old normally healing wounds. No epithelial signal was found in suction blisters, chronic ulcers or vasculitis. Stromal signal for TIMP-3 was found in all sample types in fibroblast-like cells and in occasional endothelial cells. The signal was generally most intense in the chronic wounds, and during the first days in acute wounds, locating mostly around the blood vessels. In normal skin, TIMP-3 mRNA was occasionally

expressed by fibroblasts surrounding sweat glands. *TIMP-3 protein* located in the basal keratinocytes near the wound edge in all wound types except suction blisters. As assessed by staining for *Ki-67* (Cattoretti et al, 1992), the positive cells were proliferating keratinocytes. All wound types had TIMP-3 positive fibroblast-like cells and endothelial cells, while the staining was most intense in chronic wounds. (V)

TIMP-4 protein was only produced by stromal cells near blood vessels in a few chronic wound samples. All the samples of acute wounds remained negative. (V)

Samples of normal skin generally showed no signal for MMPs and TIMPs, except for matrilysin and TIMP-3, as mentioned above. All of the samples hybridized with sense probes were negative, as well as all of the immunohistochemical control samples performed with rabbit pre-immune serum or mouse ascites fluid.

8.2. INTESTINAL ULCERATIONS (I,IV)

Table 6. Typical patterns of MMP- and TIMP- mRNA expression in ulcerative/erosive lesions caused by Crohn`s disease or ulcerative colitis.

	Crohn`s disease		*Colitis ulcerosa*	
MMPs	e	s	e	s
Collagenase-1	-	+	-	+
Collagenase-3	-	+	-	+
Stromelysin-1	-	+	-	+
Stromelysin-2	+	+	+	+
Matrilysin	+	-	+	-
Metalloelastase	-	+	-	+
TIMPs	e	s	e	s
TIMP-1	-	+	-	+
TIMP-3	-	+	-	+

MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; e, epidermal mRNA expression; s, stromal mRNA expression

Collagenase-1 mRNA was detected in nearly all intestinal ulcers in the granulation tissue in large, round cells in the bottom and the margins of the ulcer. Unlike in skin, no expression was found in the epithelium bordering the ulcer. Double labeling for collagenase-1 mRNA and *CD-68* showed that the positive cells were not macrophages and hence likely represented activated fibroblasts. The signal was very intense in samples of IBD, while the samples of gastric and duodenal ulcers displayed fewer positive cells. (I)

Collagenase-3 mRNA expression was detected in specimens of intestinal ulcers in the granulation tissue beneath the ulcer bed. The positive cells were either plump, macrophage-like or spindle,

fibroblast-like. As assessed by immunohistochemical staining for *procollagen-1*, at least part of these cells were activated fibroblasts. No signal was found in the intestinal epithelium. (IV)
Stromelysin-1 mRNA was detected in granulation tissue beneath the ulcers in large, round cells in areas corresponding to collagenase-1 mRNA expression. The expression of both enzymes was more intense in deeper ulcers. The epithelium remained negative in all the samples. (I)

Stromelysin-2 mRNA was detected in almost all the samples of intestinal ulcers in the epithelium bordering the ulcer or ruptured crypt abcesses. Immunolocalization of *Ki-67* showed that the positive cells were not of the proliferative phenotype. Staining of serial sections for *laminin-5* demonstrated co-localization of this protein and stromelysin-2. Also *matrilysin* protein co-localized with stromelysin-2 mRNA in the epithelial tip. Occasional lymphocyte- and macrophage-like cells expressed stromelysin-2 in the inflamed lamina propria. Stainings for *CD3* and *CD20* demonstrated that the majority of positive cells were not T- or B-lymphocytes, even though infiltrates of T-lymphocytes were often found close to stromelysin-2 expressing cells. (IV)

Matrilysin mRNA as well as *protein* were expressed in almost all the ulcers in the epithelium bordering the ulcer regardless of the ulcer type. Epithelial expression was also found associated with cryptitis or crypt abcesses. Normal epithelium remained constantly negative for matrilysin mRNA and protein. Immunohistochemical staining for *Ki-67* demonstrated that matrilysin-positive cells were not of the proliferative phenotype. Stainings for *laminin-1* and *fibronectin* demonstrated that the matrilysin-positive cells did not reside on a normal, intact BM. (I,IV)

Macrophage metalloelastase mRNA was abundantly expressed in all the ulcer samples investigated by plump, macrophage-like cells of the inflammatory infiltrate and the wound. Particularly in samples of ulcerative and ischemic colitis, prominent expression of HME mRNA was detected beneath the shedding epithelium. The positive cells were proven to be macrophages by immunohistochemical staining for *CD68*. *Type IV collagen* staining demonstrated continuity of the basement membrane underneath the shedding epithelium. (IV) In the skin, only a few chronic ulcers had HME mRNA positive, plump, macrophage-like cells in the vicinity of blood vessels (Vaalamo et al, unpublished results).

TIMP-1 mRNA was detected in all investigated ulcer samples. Intense signal was located in the granulation tissue at the base of the ulcer while no expression was found in the epithelium. (I)

TIMP-3 mRNA was abundantly expressed in intestinal ulcer samples. A great number of macrophage- and fibroblast-like cells of the inflamed lamina propria and granulation tissue of the ulcer bed expressed TIMP-3 mRNA. Also, cells around the blood vessel lumen were often positive for TIMP-3 mRNA. At least part of these cells stained immunohistochemically with CD 31 and were thus proven to be endothelial cells. An occasional signal for TIMP-3 mRNA was also detected in samples of normal intestine around the blood vessels and in stromal cells. (IV)

Samples of histologically normal intestine were generally negative for MMPs and TIMPs, except for TIMP-3, as mentioned above. All of the samples hybridized with sense probes were negative, as well as all of the immunohistochemical control samples performed with rabbit pre-immune serum or mouse ascites fluid.

8.3. EXPERIMENTAL MURINE INTESTINAL ANASTOMOSES (IV)

Stromelysin-2 mRNA was expressed in the granulation tissue of rat ileal anastomoses from day 4 at least until day 14. *Collagenase-3* was also expressed in the granulation tissue in plump, macrophage-like cells in 3-, 4-, 7- and 14-day samples. No signal was found in the epithelium.

8.4. EXPERIMENTAL PIGLET WOUNDS (II)

As in human wounds, *collagenase-1*, *uPa* and *TIMP-1 mRNAs* were expressed in basal keratinocytes bordering the ulcer bed, and no expression was found when the re-epithelialization was complete after day 5.

8.5. HUMAN SKIN FIBROBLAST CULTURE (III)

Fibroblasts cultured on plastic as monolayer or fibroblasts stimulated with PMA, TGF-β or IL-1β showed no signal for *collagenase-3 mRNA* after 48 hours. Culturing dermal fibroblasts inside a collagen gel for 48 hours resulted in clearly detectable levels of two collagenase-3 transcripts (2.0 and 2.5 kb). In the same cells, expression of *collagenase-1 mRNA* was detected in monolayer, and the level of collagenase-1 mRNA was markedly enhanced when the cells were cultured inside a collagen gel.

9. DISCUSSION

In this study, the expression patterns of matrix metalloproteinases of the collagenase and stromelysin subgroups, and their specific inhibitors TIMPs, were investigated in human cutaneous and intestinal wound samples using *in situ* hybridization and immunohistochemistry. *In situ* hybridization is a relevant method for investigating the cellular origin of MMPs and their inhibitors, since most MMPs are transcriptionally regulated i.e. the mRNA accumulates within the cell to detectable levels following a certain stimulus, whereas the protein is often secreted from the cells shortly after synthesis (Rodgers et al, 1994; Fini et al, 1996). However, these methods do not reveal whether these enzymes are activated or not. Due to the general lack of immunohistochemical antibodies detecting only the activated MMP form, *in situ* zymography on tissues or Westerns on wound fluids or tissue would be the most suitable assays for that purpose.

9.1. COLLAGENASES-1 AND –3 AND STROMELYSINS-1 AND -2 IN CUTANEOUS WOUND HEALING (II, III)

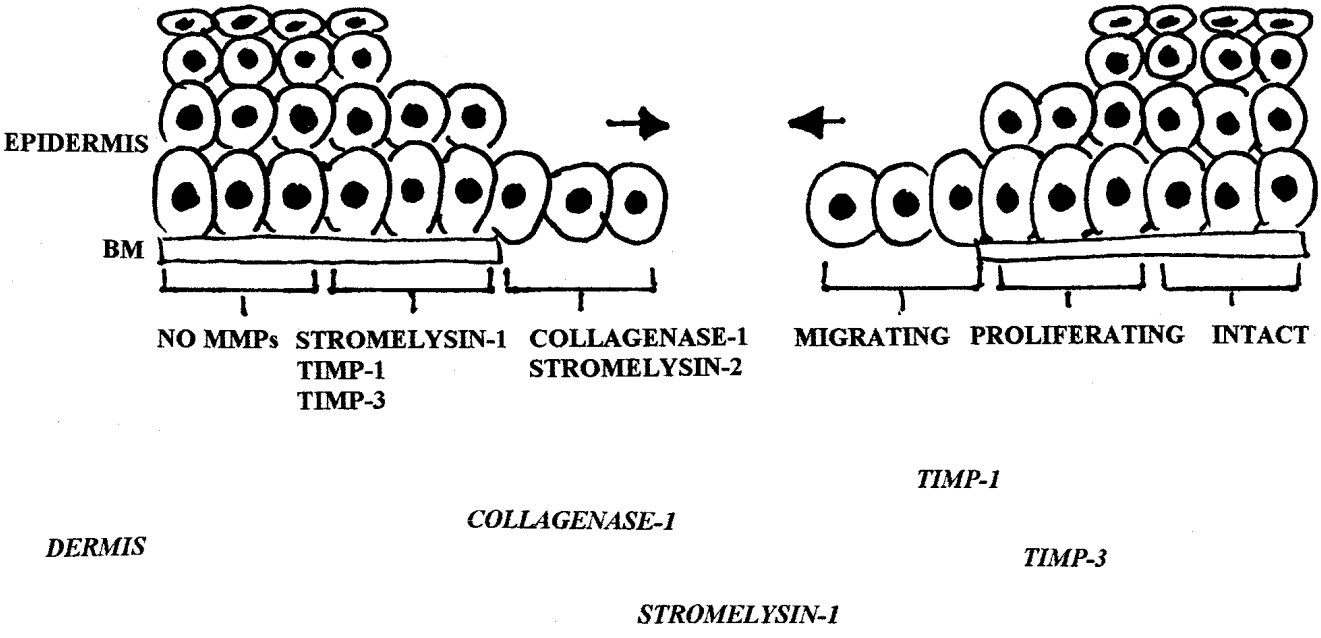

Figure 5. Collagenase-1, stromelysin-1 and -2, and TIMP-1 and -3 mRNA expression in a normally healing human wound. BM, basement membrane. Modified from Pilcher et al, 1998.

Our results on epithelial *collagenase-1* expression, limited to basal keratinocytes bordering normally healing wounds and chronic venous ulcers (II; III), are in agreement with the data in rheumatoid, diabetic, and decubitus ulcers, in ulcerated pyogenic granulomas, and in normally healing and burn wounds obtained by us and others (II; Saarialho-Kere et al, 1992; Saarialho-Kere et al, 1993a; Saarialho-Kere et al, 1993b; Stricklin et al, 1993; Inoue et al, 1995). The expression of collagenase-1 in wound edge epithelium is a constant finding, although the pathobiology of various chronic ulcer types differs from each other. In normally healing wounds collagenase-1 is expressed during the first postoperative day, and in *in vitro* wounds, as early as 4 hours after wounding (II; III; Inoue et al, 1995). The same applies to cell culture conditions, where migrating keratinocytes in contact with type I collagen express collagenase-1 at least after 24 hours of culturing (Pilcher et al, 1997). Shortly after complete re-epithelialization, no traces of collagenase-1 mRNA are found in keratinocytes (II; Stricklin et al, 1993; Inoue et al, 1995). A similar pattern of expression encountered also in healing rat and mouse skin wounds as well as in porcine burn- and incisional wounds (II; Stricklin et al, 1994b; Okada et al, 1997; Madlener et al, 1998) suggests a major role for this enzyme in dermal wound repair.

An interesting theory on the induction and role of collagenase-1 in keratinocyte migration has recently been presented by Pilcher et al (1997). Contact of keratinocytes to interstitial collagen leads to high affinity binding of integrin $\alpha 2\beta 1$ to collagen. This induces expression of collagenase-1, which degrades interstitial collagen into ¼ and ¾ fragments. These fragments denature in physiological temperature to gelatin, losing the ability to bind to integrin $\alpha 2\beta 1$ (Messent et al, 1998). The liberated keratinocyte is then able to extend and rebind to intact collagen further on the wound bed. Once the contact with fibrillar collagen is abolished by completion of re-epithelialization, collagenase-1 expression is shut off (II; III; Saarialho-Kere et al, 1993b; Stricklin et al, 1993; Inoue et al, 1995). While altered cell-ECM contacts may result in the inhibition of collagenase-1 expression, modulation of the intracellular calcium may provide for the signal to stop collagenase-1 secretion in keratinocytes (Sudbeck et al, 1997).

Although *in situ* hybridization is not a quantitative technique, we can state that the number of both epithelial and stromal collagenase-1 positive cells was higher in chronic than normally healing wounds (II, III). Similarly, elevated collagenase-1 activity has been detected in chronic wounds (Weckroth et al, 1996; Barone et al, 1998). Even though many human studies confirm the important physiological role of collagenase-1 in keratinocyte migration (Saarialho-Kere et al, 1993b; Stricklin et al, 1993; Pilcher et al, 1997), transgenic mice expressing high levels of human collagenase-1 in the epidermis suffer from delayed wound healing due to derangements in re-epithelialization (di Colandrea et al, 1998). Also, the level of active collagenase-1 may vary; tissues and fluids from normally healing wounds contain almost exclusively inactive forms of the enzyme, while fluids from non-healing ulcers possess significant amounts of the active form of collagenase-1 (Nwomeh et al, 1999). These results indicate that the homeostasis of active collagenase-1 is critical for keratinocyte migration to succeed.

A member of the serine proteases, urokinase plasminogen activator (uPA), is expressed by migrating keratinocytes in both normally healing and chronic wounds (II; Grøndahl-Hansen et al, 1988). The main task for plasminogen activation cascade during wound healing is probably fibrin degradation, since wounds in plasminogen-deficient mice have impaired fibrin resolution and heal poorly, while wounds in mice deficient in both plasminogen and fibrin heal as quickly as wounds in normal mice (Bugge et al, 1996; Rømer et al, 1996). Most probably, plasmin also activates MMPs such as collagenase-1 and stromelysin-2 in the wound edge (see Woessner, 1991). Although members of both MMP- and serine protease families appear essential to keratinocyte migration, the

migration proceeds, although slowly, with either plasminogen or MMP-activity excluded (Lund et al, 1999). Keratinocyte locomotion is ceased only if mice lack both MMP activity and plasminogen (Lund et al, 1999). These findings suggest that MMPs and serine proteases can perform each others` tasks during wound repair.

Collagenase-1 expression is not limited to the epithelium. Stromal fibroblast-like as well as macrophage-like cells express collagenase-1 in all types of wounds examined (II; III; Saarialho-Kere et al, 1992; Saarialho-Kere et al, 1993b; Stricklin et al, 1993). It is tempting to speculate that moderate expression of collagenase-1 in normally healing wounds is a part of the normal, reparative process. The prolonged presence of high amounts of collagenase-1 in chronic dermal wounds, and even in conditions preceding chronic ulcers, may implicate excess proteolysis leading to disturbed healing of the lesions (II; III; Weckroth et al, 1996; Herouy et al, 1998). A recent study by Nwomeh et al (1999) suggests that at least in chronic venous ulcer fluids, neutrophil collagenase is the predominant collagenase. We have not assessed the expression of this enzyme in wounds, but its origin most likely are the polymorphonuclear leukocytes, which are more or less abundant in chronic wounds (Herrick et al, 1992). During the inflammatory phase of acute wound healing, the neutrophils are the first cells to arrive to the injured area (see Clark, 1995), which makes it probable that collagenase-2 liberated from their granules would be responsible for initiating collagenolysis in wounds. This neutrophilic reaction may persist too long in chronic wounds, and together with the lack of activated macrophages (Moore et al, 1997), may lead to altered cytokine profiles and excessive MMP expression.

The third MMP capable of degrading fibrillar collagens, *collagenase-3* (Freije et al, 1994; Knäuper et al, 1996), has a different pattern of expression in wounds than collagenase-1. Its expression was never detected in the epithelium of any wound type, nor in the stroma of normally healing wounds (III). Contrasting acute dermal wounds, stromal fibroblasts are able to express MMP-13, when acute oral wounds heal (Ravanti et al, 1999b). This may reflect the different cellular environments of dermal and mucosal fibroblasts, and imply that studies on mucosal healing cannot always be directly extrapolated to dermal wound healing. Interestingly, collagenase-3 mRNA is expressed by inflamed gingival keratinocytes protruding towards underlying connective tissue. These MMP-13 positive cells colocalize with laminin-5 (Uitto et al, 1998), and are detected in a localization similar to gelatinase A in inflamed oral mucosa (Mäkelä et al, 1999). Thus, gelatinase A and collagenase-3 may be operational in oral wound healing and in pathological epithelial migration, while collagenase-1 assists in keratinocyte migration during dermal wound repair (Mäkelä et al, 1999). This is supported by our findings on collagenase-3 in invasive carcinomas of the skin and the mucosa, but not in the epithelium of healing cutaneous wounds (III; Airola et al, 1997; Johansson et al, 1997c; Johansson et al, 1999).

In the skin, collagenase-3 mRNA expression was detected only in fibroblast- and macrophage-like cells in fibrotic areas of chronic venous ulcers deeper in the wound bed than collagenase-1 mRNA, suggesting distinct roles for these enzymes (III). At least part of the collagenase-3 positive cells were fibroblasts, as assessed by staining for procollagen-1 (III). These fibroblasts may be stimulated to express collagenase-3 by contact with surrounding collagenous matrix mediated by integrin receptors $\alpha1\beta1$ and $\alpha2\beta1$, based on the following findings: 1) normal human skin fibroblasts cultured inside a three-dimensional collagen-gel, but not when grown in monolayer, express collagenase-3 (III) and 2) the induction generated by three-dimensional collagen is mediated by integrin receptors $\alpha1\beta1$ and $\alpha2\beta1$ (Ravanti et al, 1999a). Since collagenase-3 has a wider variety of substrates than collagenase-1, being also able to degrade e.g. gelatin (Knäuper et al, 1996), it may participate in the remodeling of the immature collagenous matrix by degrading fibrillar collagens

and their cleavage products, as well as other connective tissue components. Furthermore, collagenase-3 activates progelatinase B in a two-step cleavage mechanism *in vitro* (Wysocki et al, 1993; Bullen et al, 1995; Knäuper et al, 1997), and may activate this enzyme also in chronic ulcers *in vivo*. MT1-MMP activates both collagenase-3 and gelatinase B, yet by distinct mechanisms (see Murphy et al, 1999). MT1-MMP, gelatinase B and collagenase-3 are expressed concomitantly at least in rat wounds (Okada et al, 1997), and vulvar squamous cell carcinomas (Johansson et al, 1999), and all these enzymes can be involved in complex activation cascades of the wound environment.

In our dermal wound samples, *stromelysin-1* was expressed by keratinocytes distinct from but adjacent to the migrating front of keratinocytes, in part of both normally healing and chronic wounds (II). Due to its location stromelysin-1 is not likely to participate in keratinocyte migration. Rather, it may participate in the remodeling of the newly formed basement membrane. Stromelysin-1 is expressed in proliferating keratinocytes that already have a BM beneath them, composed of the stromelysin-1 substrates laminin and type IV collagen (II; Juhasz et al, 1993; Wilhelm et al, 1987). Also tenascin, a glycoprotein abundant in healing wounds, is readily degraded by stromelysin (see Imper & van Wart, 1998). During wound healing, tenascin mRNA is expressed by basal keratinocytes, resulting in an intense staining of this glycoprotein in the papillary dermis underneath the incomplete BM, as assessed by staining of heparan sulphate and laminin (Aukhil et al, 1995; Latjinhouwers et al, 1996; Vaalamo et al, unpublished results). Stromelysin-1 may also act in removing excess tenascin after completion of migration, when its cell-spreading activities are no longer needed. The activity of stromelysin-1 may be controlled by TIMP-1 and –3 which in acute wounds are expressed in areas partly colocalizing with stromelysin-1 expression (II; V).

As shown in this study for the first time, *stromelysin-2* mRNA is expressed in migrating keratinocytes in both acute and chronic skin wounds, as well as in full-thickness murine wounds (II; Madlener et al, 1996). Wounding of the skin results in transition of stationary keratinocytes into migrating cells, and these cells start to actively produce laminin-5 (Larjava et al, 1993a; Pyke et al, 1994). Production of this protein partly co-localizes with stromelysin-2 expression (II; Vaalamo, unpublished results; Larjava et al, 1993a). Laminin-5 has been shown either to stimulate (Zhang & Kramer, 1996), or to inhibit epithelial cell movement (O´Toole et al, 1997). Furthermore, specific cleaveage of laminin-5 by gelatinase A induces migration of breast epithelial cells (Giannelli et al, 1997). In dermal wounds, the expression and activity of gelatinase A is rather weak (Salo et al, 1994; Young & Grinnell, 1994). Since wide-spectrum proteinase stromelysin-2 is expressed in approximity or co-inciding with laminin-5 production, it is tempting to speculate that it participates in degradation of this BM component in healing wounds, and possibly accelerates re-epithelialization. However, the capacity of stromelysin-2 to degrade laminin-5 remains uninvestigated to our knowledge, and needs to be determined. Fibronectin, which is abundant in the granulation tissue, provides another candidate target for stromelysin-2 (Clark et al, 1992; Juhasz et al, 1993). However, recent studies suggest that at least in chronic wounds, neutrophil elastase is the proteinase responsible for excessive degradation of fibronectin (Rao et al 1995; Grinnell & Zhu, 1996), and may perform this task in normally healing wounds as well.

9.2. TISSUE INHIBITORS OF METALLOPROTEINASES-1, -2, -3 AND -4 IN CUTANEOUS WOUND REPAIR (II,V)

In this study, we found *TIMP-1* and *TIMP-3* mRNA expression only in the epithelium of normally healing wounds (II; V). Contrasting this, TIMP-3 protein was widely expressed by proliferating keratinocytes in both wound types. This discrepancy may be explained by mRNA levels that are too low in chronic wounds to be detected by *in situ* hybridization. TIMP-1 and –3 are regulated in response to cell-ECM interactions and by various cytokines; e.g. laminin-1 inhibits TIMP-1 expression while collagen type I stimulates it in cultured keratinocytes (Petersen et al, 1990). Furthermore, TGF-β stimulates the expression of TIMP-3 at least in primary human keratinocytes (Airola et al, 1998). TIMP-1 and –3 expression induced in wound edge keratinocytes may be needed in 1) the protection of the newly formed basement membrane from degradation by epithelial MMPs, e.g. stromelysin-1 (II), 2) in the regulation of keratinocyte proliferation due to their growth promoting activities (see Gomez et al, 1997), or 3) the protein released from the cells into the matrix may be needed to inactivate excess MMPs produced by the leading edge of keratinocytes. *TIMP-2* protein locates beneath the migrating front of keratinocytes, and it is found in greater amounts in acute wounds than in chronic ones (V). Whereas TIMPs-1 and –3 may act chiefly in neutralizing secreted collagenase-1, TIMP-2 may neutralize the gelatinases, according to their *in vitro* preferences (Howard et al, 1991; Apte et al, 1995).

Expression of TIMPs is not limited to epithelial structures. In addition to TIMP-2 protein, expression for TIMP-1 mRNA, and TIMP-3 mRNA and protein was detected in the stromal compartment of healing wounds. Stromal expression of these enzymes may be needed in the inactivation of stromal MMPs such as collagenase-1 and stromelysin-1 (II; III; Stricklin et al, 1993), and collagenase-3 in chronic ulcers (III). Localization of TIMPs-1 and –3 surrounding blood vessels suggests a role in regulating wound angiogenesis (Johnson et al, 1994; Anand-Apte et al, 1997). *TIMP-4* is the most recently cloned of the TIMPs (Greene et al, 1996). It exhibits a restricted pattern of moderate expression in human tissues such as the heart, kidney, placenta, colon and testes (Greene et al, 1996). Accordingly, we found TIMP-4 protein only in sporadic activated fibroblast-like cells of chronic wound stroma. Thus, the role of TIMP-4 appears a minor one in dermal wounds, compared to other TIMPs (II; V).

Tissue inhibitors of metalloproteinases are considered to be the major inhibitors for MMPs. However, unspecific inhibitors such as alpha 2-macroglobulin may also participate in the regulation of MMPs in wound environment, since collagenase-1-alpha 2-macroglobulin complexes have been detected in both normally healing burn wounds and chronic venous ulcers (Grinnell et al, 1998). Our studies indicate that the relative lack of local inhibitors of MMPs, TIMPs-1, -2, and –3, in chronic ulcers may lead to inadequate activation of MMPs, with subsequent digestion of the ECM, and perhaps, delayed migration and disturbed wound healing.

Excluding the suction blisters, the wound samples we used in our studies were from elderly people (II; III; V). Aging alters the inflammatory response to injury; wounds of the aged show an early marked increase in the neutrophil response, and delayed monocyte/macrophage and lymphocyte appearance together with alterations in the inflammatory and endothelial cell adhesion molecule profiles (Ashcroft et al, 1998). The age-related increase in gelatinases A and B with gelatin degradation and proteinase activity is detected in acute wounds, while collagenase-1 and stromelysin-1 profiles do not differ in the young and the aged (Ashcroft et al, 1997a). TIMP-1 and -

2 mRNA levels in wounds of the young are elevated between 3-21 days, while the aged have a constant basal level expression of TIMP-1 and –2 (Ashcroft et al, 1997b). Our results on MMP-expression in normally healing wounds represent the events in wounds of the aged, and may differ in some aspects from normally healing wounds in the young. However, the fact that both the acute and the chronic wound samples examined were from people over 50 years of age, makes comparing of these wound types more reliable.

9.3. COLLAGENASES-1 AND –3, STROMELYSINS-1 AND –2, MATRILYSIN AND MACROPHAGE METALLOELASTASE IN INFLAMMATORY BOWEL DISEASES (I, IV)

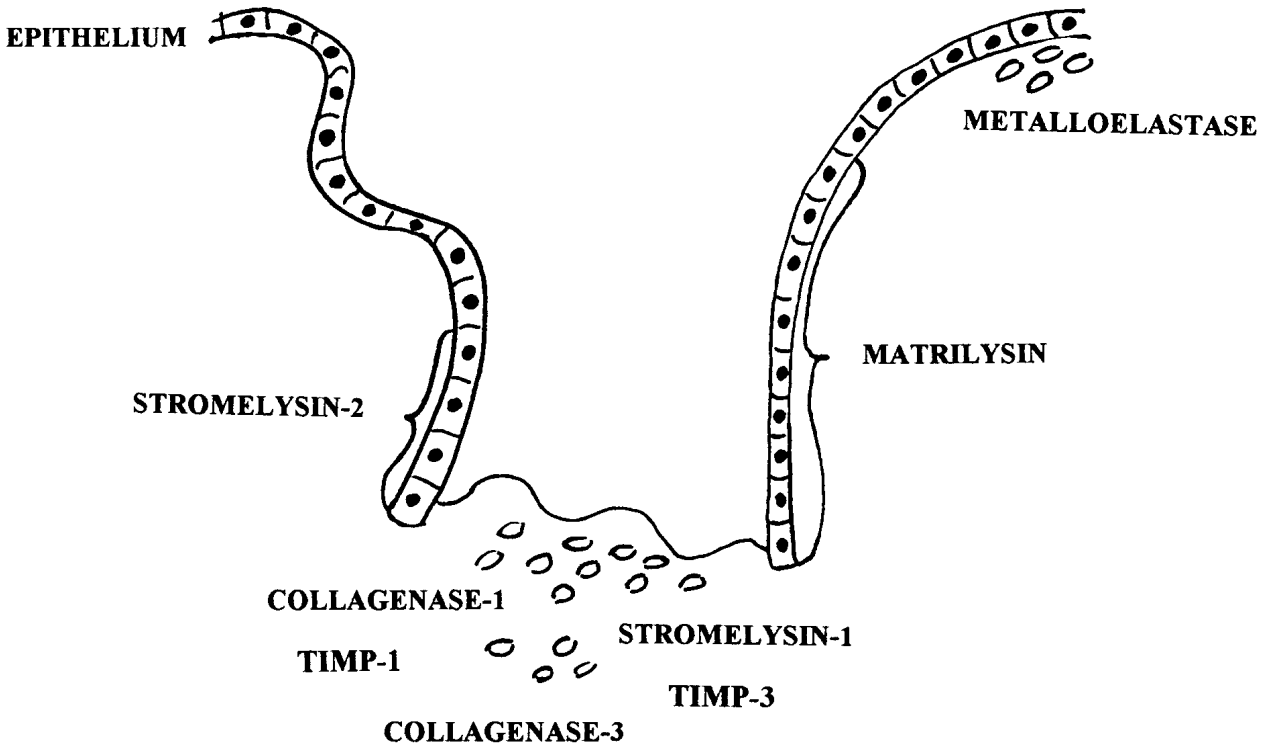

Figure 6. Collagenases-1 and -3, stromelysins-1 and -2, matrilysin, macrophage metalloelastase, and TIMPs-1 and -3 mRNA expression in IBD with intestinal ulceration.

Contrasting the only previous study demonstrating sporadic collagenase-1 protein expression in IBD (Bailey et al, 1994), we detected abundant expression of *collagenase-1* mRNA in the stroma of IBD lesions (I). The signal located beneath the ulcers in plump cells, which were most probably activated fibroblasts, since double labeling showed distinct location of collagenase-1 mRNA and the

macrophage marker CD-68 (I). Not only was collagenase-1 mRNA expressed in IBD; a majority of chronic intestinal ulcer samples showed expression for *collagenase-3* mRNA in the granulation tissue, yet in fewer cells than collagenase-1 (I; IV). Unlike in skin, the signal was not confined to fibrotic areas or cells surrounding blood vessels. Rather, deeply penetrating ulcers showed diffuse signal in fibroblast and macrophage-like cells of the granulation tissue (IV). In both dermal and intestinal ulcers, at least part of the positive cells were shown to be fibroblasts, while we could not demonstrate any macrophage being responsible for the expression of collagenase-3 mRNA (III; IV). The epithelium remained negative for both collagenase-1 and collagenase-3 in the gut (I; IV).

Inflammation in the intestine may be responsible for the induction of MMPs in IBD. Chronic immune activation outside as well as inside the lamina propria has been suggested in these diseases (Pallone et al, 1987). Strong evidence for T-cell mediated damage of the intestine is obtained from studies with fetal ileal organ explants (Pender et al, 1996; Pender et al, 1997); collagenase-1, gelatinase A, and stromelysin-1 are produced in response to T-cell activation, and they are upregulated in the mesenchymal cells by IL-1β and TNF-α. Elevated levels of IL-1β and TNF-α have been shown in IBD *in vivo* as well (Ligumsky et al, 1990; Cappello et al, 1992; Murch et al,1993). Furthermore, recombinant stromelysin-1 added to mucosal explants results in tissue destruction within 24 hours, while synthetic inhibitor of MMPs reduces mucosal damage caused by T-cell activation (Pender et al, 1997). This provides evidence that activation of lamina propria T-cells responding to luminal antigens may lead to tissue damage by MMPs. T-cells may not only mediate tissue damage by altered cytokine/growth factor profiles, but are also able to express MMPs such as stromelysin-2 and gelatinase B (Conca & Wilmroth, 1994; Johnatty et al, 1997). In our studies, IBD specimens showed intense mRNA expression for collagenase-1, stromelysin-1, and HME, and moderate expression for collagenase-3 by the stromal cells (I; IV). While the collagenases may be responsible for the digestion of fibrillar collagens, stromelysin-1 and HME could mediate the loss of lamina propria glycosaminoclycans such as chondroitin and dermatan sulphates (Murch et al, 1993; Pender et al, 1996; see Chandler et al, 1997; see Wilson 1998). Altogether, these results indicate that T-cells may have an important role in mediating tissue damage by MMPs in IBD *in vivo*, too. Mast cells, which are particularly numerous in the intestinal wall (see Pope, 1998), release serine proteinases chymase and tryptase and may contribute to the intestinal damage by activating stromal MMPs collagenase-1 and stromelysin-1 (Lees et al, 1994).

As in dermal wounds, *stromelysin-2* mRNA was detected in migrating intestinal epithelium bordering the ulcers, erosions and ruptured crypt abscesses, partly co-localizing with laminin-5 (I; IV). KGF is a cytokine capable of inducing stromelysin-2 at least in cultured HaCat keratinocytes, and its mRNA and protein expression is upregulated in both murine dermal wounds and, up to 150-fold, in IBD (Werner et al, 1992; Brauchle et al, 1996). This potent mitogen for both keratinocytes and gastrointestinal epithelial cells may contribute to stromelysin-2 induction in both dermal and intestinal wounds in humans. In IBD, we detected stromelysin-2 in areas of disrupted epithelium (IV), and analogously, expression of KGF was particularly high in areas characterized by epithelial damage in IBD (Brauchle et al, 1996).

Like collagenase-1 in dermal wounds, *matrilysin* was detected in migrating intestinal epithelial cells (I; IV). Both matrilysin mRNA and protein were expressed by the wound edge intestinal epithelium, while in the skin, only normal glandular epithelium expresses matrilysin, with no induction during wound healing (II; Saarialho-Kere et al, 1995). This may reflect differences in the composition of the ECM in these organs. While dermis is dense and rich in fibrillar collagens, the lamina propria of the intestine is loose and cellular, and there is no such need for degradation of fibrillar collagen as in the skin. Our results also support the previous findings of matrilysin mainly in cells and tissues of

secretory epithelial origin (see Wilson et al, 1998). In contrast to findings in mice (Wilson et al, 1995), matrilysin is not expressed in intact intestinal mucosa (I; IV). In mice, matrilysin co-localizes in the Paneth cell granules with bactericidal cryptidins, and *in vitro* it is able to process cryptidin precursors (Wilson et al, 1999). Thus, it may function in intestinal mucosal defense in mice. We did not assess matrilysin in normally healing intestinal wounds due to the lack of proper samples. This makes it difficult to judge whether matrilysin really is needed for normal wound repair, without underlying disease and inflammation. Matrilysin may also play a role in the pathogenesis of inflammatory bowel disease, since it is able to process proTNF-α, an inflammatory mediator found in IBD tissues (Cappello et al, 1992; Murch et al, 1993; Chandler et al, 1996).

Our study provides the first evidence of matrilysin in human intestinal epithelium without malignant progression (I). Earlier studies focused on the role of matrilysin in both premalignant adenomas as well as colon and gastric carcinomas, with no expression in normal or inflamed intestine (McDonnel et al, 1991; Newell et al, 1994; Yamamoto et al, 1994). It has been suggested, that gastric carcinomas use integrin α6β4, laminin-5, and laminin-1 as the machinery of adhesion and invasion (Tani et al, 1996). Among these, β4-integrin subunit is degraded by matrilysin *in vitro* (von Bredow et al, 1997). The anchoring complex components α6 and β4 integrin subunits are a part of normal intestinal epithelium as well (Leivo et al, 1996). Since expression of matrilysin by migrating cells of the intestinal epithelium was a constant finding (I; IV), it is tempting to speculate that both benign and malignant intestinal epithelial cells use matrilysin to detach from the anchoring complex by degrading β4-integrin subunit, when migrating.

EGF is involved in protection as well as healing of gastrointestinal mucosa (Konturek et al, 1991). It may, together with TGF-α, stimulate cell proliferation and DNA synthesis in the healing of gastric lesions, and protect the intestine against ethanol or stress induced damage in rats (Konturek et al, 1992). Other cytokines that have been shown to accelerate healing of GI-lesions include PDGF and TGF-β (Mustoe et al, 1990; see Jones et al, 1999). Culturing enterocytes on collagen stimulates migration more than laminin or fibronectin (Basson et al, 1992a; Basson et al, 1992b). However, EGF and TGF-β stimulate cultured enterocytes migration only on laminin (Basson et al, 1992a; Basson et al, 1992b). Different effects on migration may reflect specific repair processes of the intestine; namely restitution and repair by reepithelialization and proliferation. These growth factors may also influence the expression of matrilysin and stromelysin-2 in the migrating cells. It would be interesting to know whether these enzymes are involved in both repair processes. Unfortunately, we were not able to determine any areas with only restitution going on. Therefore we can only suggest that in the intestine, cells migrating over the connective tissue express matrilysin and stromelysin-2, and require these enzymes probably for degradation of ECM/BM components (I; IV).

No expression of matrilysin was detected in the connective tissue compartments of dermal or intestinal ulcers (I; II). However, *macrophage metalloelastase* was expressed intensively by macrophage-like cells in IBD (IV). All the samples of IBD were positive for metalloelastase, and the positive cells located in two distinct areas: 1) underneath the shedding epithelium and 2) within the macrophage-rich zone of the inflamed connective tissue. HME has a broad substrate specificity with the ability to degrade BM components such as type IV collagen, laminin and fibronectin (see Chandler et al, 1997). We suggest a role for this macrophage-derived enzyme in the shedding of intestinal epithelium, possibly preceding formation of erosion/ulcer during the course of IBD (IV). Deeper in the stroma, this enzyme may also aid in macrophage migration, a process in which it has previously been implicated (Shipley et al, 1996).

9.4. TISSUE INHIBITORS OF METALLOPROTEINASES-1 AND –3 IN INFLAMMATORY BOWEL DISEASE (I,V)

Like in chronic cutaneous wounds (II; V), no TIMP-1 nor TIMP-3 mRNAs were detected in the epithelium of chronic intestinal ulcers (I; IV). No TIMP transcripts were detected in the epithelium even in intestinal samples with histologically active re-epithelialization (IV). When comparing the expression of TIMP-3 in cutaneous and intestinal wounds, one must bear in mind its tightly scheduled expression; only 3-5 days old skin wounds exhibited epithelial transcripts for TIMPs (I; V). Determining the duration of intestinal ulcers and erosions is practically impossible, and clinical course of the IBD suggests rather long-lasting injuries.

The inflammatory infiltrate of the chronic intestinal ulcers and erosions showed strong signal for both TIMP-1 and TIMP-3 (I; IV). The expression of these inhibitors co-localized partly with that of MMPs such as collagenases-1 and –3 and stromelysin-1 (I; IV). TIMPs may be upregulated in the intestine to prevent damage caused by the MMPs. Various cytokines and growth factors of the inflamed intestine may trigger TIMP production, and it is tempting to speculate that factors beneficial to intestinal wound healing, such as IL-10 and EGF (see Anand-Apte et al, 1996; Pender et al, 1998a; see Jones et al, 1999) would mediate the upregulation of TIMPs, and in this way limit tissue destruction by MMPs.

On one hand, the location of TIMP-3 mRNA around blood vessels in IBD proposes a role for it in stabilizing vessel walls, since it has antiangiogenic properties (Anand-Apte et al, 1997). On the other hand, overexpression of TIMP-3 in rat vascular smooth muscle cells promotes apoptosis (Baker et al, 1998) which does not exclude an alternate role for TIMP-3 in mediating apoptosis of vascular cells with subsequent vessel regression.

Inflammatory bowel disease lesions have elevated levels of TNF-α (Murch et al, 1993; Ligumsky et al, 1990), which may mediate tissue damage by accelerating the inflammatory response. The p55 TNF receptor immunoadhesin prevents TNF-α-mediated MMP-production and tissue damage of the intestine (Pender et al, 1998b). Conversion of this cytokine from proform into active form can be prevented by TIMP-3, an inhibitor of TACE (TNF-α converting enzyme) (Amour et al, 1998). Thus, TIMP-3 may inhibit proteolysis not only by local inhibitory acitivity, but also by preventing MMP-production.

9.5. MATRIX METALLOPROTEINASES IN ANIMAL MODELS

Even though MMPs have been implicated in developmental and disease processes, their actual role remains unraveled. Many *in vitro* studies have elucidated their substrate specificities and activities, but none of these results have been proven *in vivo* (see Woessner, 1998). Therefore, it can only be suggested that they degrade ECM molecules also *in vivo*. To resolve the actual consequences of MMP activity in tissues, various MMP-knockout mice have been generated (see Shapiro, 1998). Indeed, different *in vivo* tasks for MMPs can be suggested according to these studies. For example,

macrophages from HME-deficient mice fail to penetrate basement membranes (Shipley et al, 1996), and gelatinase A and matrilysin deficiencies inhibit tumor growth (Itoh et al, 1998; Wilson et al, 1997), indicating roles for these MMPs in macrophage migration and tumor cell invasion, respectively. However, wound healing has only been investigated in mice deficient in stromelysin-1 (Bullard et al, 1999). These mice display no disorders in migration or reepithelialization, but suffer from impaired wound contraction (Bullard et al, 1999). The viability and rather normal development of many MMP-knockout mice suggest that many of the tasks of a single enzyme can be performed by other proteinases, due to overlapping degradative activities. This functional overlap is demonstrated by impaired wound healing in mice with either plasminogen deficiency or galardin-blocked MMP-activity, but a total failure to heal in mice with both plasminogen deficiency and galardin-treatment (Lund et al, 1999).

Many of the studies cited here were performed with mice, rats, rabbits or pigs (Stricklin et al, 1994b; Fini et al, 1996; Madlener et al, 1996; Okada et al, 1997; Madlener et al, 1998). We too performed part of our work with animals: collagenase-1 and TIMP-1 mRNA expression in incisional pig wounds confirmed the findings in humans (II), as was the case in burn wounds of pigs and humans (Stricklin et al, 1993; Stricklin et al, 1994b). In normally healing rat wounds, MMPs such as collagenase-3, gelatinase A, MT1-MMP and stromelysin-1 were coordinately expressed, and MT1-MMP was shown to activate progelatinase A in the wound stroma (Okada et al, 1997). These results partly correspond to those of humans. Of interest was the expression of collagenase-3 in the migrating epithelium, which did not occur in normally healing human skin wounds (Okada et al, 1997; III). This may be explained by rat collagenase-3 considered as the counterpart of human collagenase-1 (Knäuper et al, 1996). Similarly as in humans, no matrilysin was found during rat skin wound healing (Okada et al, 1997). Expression of stromelysin-2 in wound healing in mice, and lack of the enzyme in rat wounds, as well as different MMP-expression patterns in rat and rabbit corneal wound healing, provide examples of MMP variations between rodents (Madlener et al, 1996; Fini et al, 1996; Okada et al, 1997). In order to determine stromelysin-2 and collagenase-3 expression in normally healing intestinal wounds, we used samples of rat ileal anastomosis (IV). These samples provided somewhat unexpected results: stromelysin-2 mRNA expression did not localize in the migrating epithelium, but in the granulation tissue, as the expression for collagenase-3 mRNA (IV). In mice, matrilysin was constitutively expressed by Paneth cells in crypts of normal small intestine (Wilson et al, 1995), which was not supported by our work in human intestine (I; IV). Rats with acetic acid induced gastric ulcers displayed a rapid elevation in collagenase-1 and gelatinase B levels, with gradual return to control values during the healing phase (Baragi et al, 1997). Colonic anastomoses resulted in elevated MMP and TIMP-1 expression confined to the suture line, while anastomoses combined with ischemia or colonic obstruction resulted in a more widespread expression of MMPs and TIMP-1 (Savage et al, 1997; Savage et al, 1998). Data on MMPs in acute human intestinal lesions would be valuable, but such samples are difficult to obtain. Altogether, these slightly varying, species dependent results indicate that even though work that has been done with animal models provides valuable information, direct conclusions to healing human wounds cannot be drawn.

10. CONCLUSIONS

Wound healing is an orderly process, during which the disrupted epithelium is restored by cell migration and proliferation, and the injured tissue is replaced by the newly deposited matrix. Most, if not all the components of the extracellular matrix and the basement membranes can be degraded by matrix metalloproteinases (MMPs). The expression of MMPs is controlled by cytokines, growth factors, oncogenes, and changes in the cell-cell and cell-matrix interactions. Tissue inhibitors of metalloproteinases (TIMPs) -1, -2, -3 and -4, are the most important inhibitors of MMPs, whose activities are required not only to prevent excessive proteolysis of tissues but also to regulate e.g. cell proliferation and angiogenesis. MMPs have been implicated in various physiological and pathological conditions that require remodeling of the extracellular matrix. In dermal wounds, they may serve a role in e.g removing the devitalized tissue, in dissecting the way for migratory cells, or in activating latent growth factors.

Based on our results, migrating keratinocytes express MMPs, collagenase-1 and stromelysin-2, during wound healing. Expression of collagenase-1 is induced rapidly after injury, possibly by the keratinocyte contact to extracellular matrix. Urokinase plasminogen activator, which is expressed by the migrating keratinocytes as well, may lead to plasminogen activation cascade and degradation of the fibronectin and fibrin-rich provisional matrix, together with activation of collagenase-1 and stromelysin-2. Collagenase-1 and stromelysin-1 participate also in the remodeling of the newly deposited connective tissue. Matrilysin is not involved in dermal wound healing.

TIMPs-1 and -3 are expressed by basal keratinocytes further from the migrating front than collagenase-1, where they may act in protecting the re-established basement membrane from degradation. TIMP-1, TIMP-2 and TIMP-3 are expressed also in the matrix, probably neutralizing the MMPs secreted by the epithelial as well as the stromal cells. TIMP-4 appears to play a minor role in human wound healing.

The patterns of MMPs and their inhibitors are qualitatively rather similar in acute and chronic wounds. The major differences include 1) higher quantities of e.g. collagenase-1 mRNA positive epithelial and stromal cells in chronic wounds 2) presence of collagenase-3 mRNA in the stroma of chronic, but not in acute wounds and 3) lack of TIMP-1 and TIMP-3 mRNA expression in proliferating epithelium of chronic wounds.

In active, ulcerative IBD, collagenases-1 and -3 and stromelysin-1 probably participate in matrix remodeling in the granulation tissue beneath the ulcers/erosions. Matrilysin and stromelysin-2 may be needed for the migration of intestinal epithelial cells, while macrophage metalloelastase is involved in macrophage migration. TIMPs-1 and -3 are expressed in stromal areas corresponding to collagenases-1 and -3 and stromelysin-1 expression, while similarly to chronic cutaneous wounds, epithelial cells do not express TIMPs.

In conclusion, successful wound healing is accompanied by tightly scheduled expression of metalloproteinases, their inhibitors and activators. Imbalance of the proteinases and their inhibitors may delay wound healing and result in chronic wounds. MMPs and TIMPs are also involved in both tissue damage and mucosal reparative processes during the course of inflammatory bowel diseases.

11. ACKNOWLEDGEMENTS

These studies were carried out in the Departement of Dermatology and Venereology, Helsinki University Central Hospital, during the years 1994-1999.

I wish to express my sincere gratitude to those who have contributed to this work:

Professors Annamari Ranki and Timo Reunala for providing excellent facilities for the studies.

Professor Jorma Keski-Oja for providing the facilities and the support that enabled me to fully concentrate in this work during the year 1996.

My supervisor, Docent Ulpu Saarialho-Kere, deserves my deepest gratitude for introducing me to the world of metalloproteinases, and guiding me through these studies. I admire her never-failing, productive enthusiasm in scientific work, and the ability to always be ready for discussions, despite the busy schedule.

Professor Juha Kere for friendly discussions, during which he offered useful pieces of his vast genetic knowledge.

Docent Arja-Leena Kariniemi for her invaluable dermatopathological knowlegde, and for her friendliness and interest in fields that extend beyond the everyday work.

Professor Aarne Oikarinen and Docent Matti Laato for kindly examining this thesis, and for their constructive comments on the text.

My co-authors, Docents Veli-Matti Kähäri and Pauli Puolakkainen, Professor William Parks, and Drs. Marja-Liisa Karjalainen-Lindsberg, Nina Johansson, Laura Ravanti, Pia Saarinen, Jorma Lauharanta, Miina Weckroth and Tomi Leivo, for pleasant and fruitful collaboration.

Dr. Kristiina Airola not only for her contribution to the lab work in these studies, but especially for her friendship, that has brightened up many days both in the lab and in the outside world.

My friends and colleagues Outi, Tiina, Erja, Hanna, Leena, Mikko S., Oona, Sari and Siru for the time shared at work, for invaluable help in solving various kinds of problems, and for lunch-breaks and free-time with vivid conversations.

The ladies in the lab: Alli, Anna-Mari, Birgitta, Kaija, Liisa S. and Marja for excellent technical help and Liisa L. for excellent secretarial help, and for their interest and caring during these years.

David Mauffret for revising the English language.

In addition, I wish to give all my dear friends, and their families warm hugs for making my spare time most enjoyable. Especially Mari deserves a big hug for sharing the ups and downs of both research and everyday life.

I most warmly thank my parents Leila and Juhani Keski-Säntti for all the love and support they have given to our family during these years. I thank my brothers Harri and Jouko, and their lovely families for caring, and for sharing many of the best moments of my life. Also, Mikko´s parents Pirkko and Pentti Vaalamo, sister Tuija and brother Jukka with their families, deserve my deepest thanks for the enjoyable times we have spent together.

Most of all, I thank Mikko, Emilia and Veikka for filling my life with love, precious moments and joy. Mikko has shared all these years with me, and his love and care have been the greatest support to all the aspects of my life.

This thesis has been financially supported by the Helsinki University Central Hospital Research Fund, The University of Helsinki, Finska Läkaresällskapet, the Paulo Foundation, the Sigrid Juselius Foundation, the Farmos Research Foundation, the Duodecim Foundation, the Finnish Dermatological Society, and the Dermatopathological Society.

11. REFERENCES

Ahonen M, Baker AH, Kähäri V-M: Adenovirus-mediated gene delivery of tissue inhibitor of metalloproteinases-3 inhibits invasion and induces apoptosis in melanoma cells. Cancer Res 1998, 58: 2310-2315

Airola K, Vaalamo M, Reunala T, Saarialho-Kere UK: Enhanced expression of interstitial collagenase, stromelysin-1, and urokinase plasminogen activator in lesions of dermatitis herpetiformis. J Invest Dermatol 1995, 105: 184-189

Airola K, Johansson N, Kariniemi A-L, Kähäri V-M, Saarialho-Kere UK: Human collagenase-3 is expressed in malignant squamous epithelium of the skin. J Invest Dermatol 1997, 109: 225-231

Airola K, Ahonen M, Johansson N, Heikkilä P, Kere J, Kähäri V-M, Saarialho-Kere UK: Human TIMP-3 is expressed during fetal hair development, hair growth cycle and cancer progression. J Histochem Cytochem 1998, 46: 1-11

Airola K, Karonen T, Vaalamo M, Lehti K, Lohi J, Kariniemi A-L, Keski-Oja J, Saarialho-Kere UK: Expression of collagenases-1 and -3 and their inhibitors TIMP-1 and –3 correlates with the level of invasion malignant melanomas. Br J Cancer 1999, 80: 733-743

Amour A, Slocombe PM, Webster A, Butler M, Knight CG, Smith BJ, Stephens PE, Shelley C, Hutton M, Knäuper V, Docherty AJP, Murphy G: TNF-α converting enzyme (TACE) is inhibited by TIMP-3. FEBS Lett 1998, 435: 39-44

Anand-Apte B, Bao L, Smith R, Iwata K, Olsen BR, Zetter B, Apte SS: A review of tissue inhibitor of metalloproteinases-3 (TIMP-3) and experimental analysis of its effect on primary tumor growth. Biochem Cell Biol 1996, 74: 853-862

Anand-Apte B, Pepper MS, Voest E, Montesano R, Olsen B, Murphy G, Apte S and Zetter B: Inhibition of angiogenesis by tissue inhibitor of metalloproteinase-3. Invest Ophtalmol & Vis Sci 1997, 38: 817-823

Andriessen MPM, Van Bergen BH, Spruijt KIJ, Go IH, Schalkwijk J, Van de Kerkhof PCM: Epidermal proliferation is not impaired in chronic venous ulcers. Acta Derm Venereol 1995, 75: 459-462

Apte SS, Mattei MG, Olsen BR: Cloning of the cDNA encoding tissue inhibitor of metalloproteinases (TIMP-3) and mapping of the TIMP-3 gene to chromosome 22. Genomics 1994, 19: 86-90

Apte SS, Olsen B, Murphy G: The gene structure of tissue inhibitor of metalloproteinases (TIMP)-3 and its inhibitory activities define the distinct TIMP gene family. J Biol Chem 1995, 270: 14313-14318

Ashcroft GS, Horan MA, Herrick SE, Tarnuzzer RW, Schultz GS, Ferguson MWJ: Age-related differences in the temporal and spatial regulation of matrix metalloproteinases (MMPs) in normal skin and acute cutaneous wounds of healthy humans. Cell Tissue Res 1997a, 290: 581-591

Ashcroft GS, Herrick SE, Tarnuzzer RW, Horan MA, Schultz GS, Ferguson MWJ: Human aging impairs injury-induced expression of tissue inhibitor of metalloproteinases (TIMP)-1 and -2 protein and mRNA. J Pathol 1997b, 183: 169-176

Ashcroft GS, Horan MA, Ferguson MWJ: Aging alters the inflammatory and endothelial cell adhesion molecule profiles during human cutaneous wound healing. Lab Invest 1998, 78: 47-58

Ashworth JL, Murphy G, Rock MJ, Sherratt MJ, Shapiro SD, Shuttleworth CA, Kielty CM: Fibrillin degradation by matrix metalloproteinases: Implications for connective tissue remodeling. Biochem J 1999, 112: 3549-3558

Auble C, Brinckerhoff CE: The AP-1 sequence is necessary but not sufficient for phorbol induction of collagenase in fibroblasts. Biochem 1991, 30: 4629-4635

Aukhil I, Sahlberg C, Thesleff I: Basal layer of epithelium expresses tenascin mRNA during healing of incisional skin wounds. J Periodont Res 1995, 31: 105-112

Baharestani M: The clinical relevance of débridement. In: The clinical relevance of débridement. Eds. Baharestani M, Gottrup F, Holstein P, Vanscheidt W. Springer-Verlag Berlin Heidelberg 1999, pp. 11-13

Bailey C, Hembry R, Alexander A, Irving M, Grant, Shuttleworth C: Distribution of the matrix metalloproteinases stromelysin, gelatinases A and B, and collagenase in Crohn`s disease and normal intestine. J Clin Pathol 1994, 47: 113-116

Baker AH, Zaltsman AB, George SJ, Newby AC: Divergent effects of tissue inhibitor of metalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation and death in vitro. J Clin Invest 1998, 101: 1478-1487

Baker SR, Stacey MC, Singh G, Hoskin SE, Thompson PJ: Aetiology of chronic leg ulcers. Eur J Vasc Surg 1992, 6: 245-251

Baragi VM, Qiu L, Gunja-Smith Z, Woessner JF Jr, Lesch CA, Giuglietta A: Role of metalloproteinases in the development and healing of acetic acid-induced gastric ulcer in rats. Scand J Gastroenterol 1997, 32: 419-426

Barone EJ, Yager D, Pozez AL, Olutoye OO, Crossland MC, Diegelmann RF, Cohen IK: Interleukin-1α and collagenase activity are elevated in chronic wounds. Plast Reconstr Surg 1998, 102: 1023-1927

Basset P, Wolf C, Chambon P: Expression of the stromelysin-3 gene in fibroblastic cells of invasive carcinomas of the breast and other human tissues: a review. Breast Cancer Res Treatment 1993, 24: 185-193

Basset P, Bellocq JP, Wolf C, Stoll I, Hutin P, Limacher JM, Podhajcer OL, Chenard MP, Rio MC, Chambon P: A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 1990, 348: 699-704

Basson MD, Modlin IM, Madri JA: Human enterocyte (CaCo-2) migration is modulated in vitro by extracellular matrix composition and epidermal growth factor. J Clin Invest 1992a, 90: 15-23

Basson MD, Modlin IM, Flynn SD, Jena BP, Madri JA: Independent modulation of enterocyte migration and proliferation by growth factors, matrix proteins, and pharmacologic agents in an in vitro model of mucosal healing. Surgery 1992b, 112: 299-308

Beaulieu J-F: Differential expression of the VLA family of integrins along the crypt-villus axis in the human small intestine. J Cell Sci 1992, 102: 427-436

Beaulieu J-F: Integrins and human intestinal cell functions. Front Biosci 1999, 4: 310-321

Belaaouaj J, Shipley M, Kobayashi D, Zimonjic D, Popescu N, Silverman G, Shapiro S: Human macrophage metalloelastase. J Biol Chem 1995, 270: 14568-14575

Benbow U, Brinckerhoff CE: The AP-1 site and MMP gene regulation: What is all the fuss about? Matrix Biol 1997, 15: 519-526

Bertaux B, Hornebeck W, Eisen AZ and Dubertret L: Growth stimulation of human keratinocytes by tissue inhibitor of metalloproteinases. J Invest Dermatol 1991, 97: 679-685

Birkedal-Hansen H, Moore WGI, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA: Matrix metalloproteinases: A review. Crit Rev Oral Biol Med 1993, 934-6154

Borradori L, Sonnenberg A: Structure and function of hemidesmosomes: More than simple adhesion complexes. J Invest Dermatol 1999, 112: 411-418

Brasken P: Healing of experimental colon anastomosis. Eur J Surg 1991, 566 (Suppl): 1-52

Brauchle M, Madlener M, Wagner AD, Angermeyer K, Lauer U, Hofschneider PH, Gregor M, Werner S: Keratinocyte growth factor is highly overexpressed in inflammatory bowel disease. Am J Pathol 1996, 149:521-529

Brook I, Frazier EH: Aerobic and anaerobic microbiology of chronic venous ulcers. Int J Dermatol 1998, 37: 426-428

Bugge TH, Kombrinck KW, Xiao Q, Holmback K, Daugherty CC, Danton MJ, Degen JL: Loss of fibrinogen rescues mice from pleiotropic effects of plasminogen deficiency. Cell 1996, 87: 709-719

Buisson A-C, Gilles C, Polette M, Zahm J-M, Birembaut P, Tournier J-M: Wound repair-induced expression of stromelysins is associated with the acquisition of a mesenchymal phenotype in human respiratory epithelial cells. Lab Invest 1996, 74: 658-669

Bullard KM, Lund L, Mudgett JS, Mellin TN, Hunt TK, Murphy B, Ronan J, Werb Z, Banda MJ: Impaired wound contraction in stromelysin-1 deficient mice. Ann Surgery 1999, 230: 260-265

Bullen EC, Longaker MT, Updike DL, Benton R, Ladin D, Zizheng H, Howard EW: Tissue inhibitor of metalloproteinases-1 is decreased and activated gelatinases are increased in chronic wounds. J Invest Dermatol 1995, 104: 236-240

Burgeson RE, Christiano AM: The dermal-epidermal junction. Curr Opin Cell Biol 1997, 9: 651-658

Busiek DF, Ross FP, McDonnel S, Murphy G, Matrisian LM, Welgus HG: The matrix metalloprotease matrilysin (PUMP) is expressed in developing human mononuclear phagocytes. J Biol Chem 1992, 13: 9087-9092

Butler GS, Butler M, Atkinson SJ, Will H, Tamura T, Schade van Westrum S, Crabbe TG, Clements J, d´Ortho M-P, Murphy G: The TIMP-2 membrane type I metalloproteinase ”receptor” regulates the concentration and efficient activation of progelatinase A. A kinetic study. J Biol Chem 1997, 273: 871-880

Campbell EJ, Cury JD, Shapiro SD, Goldberg GI, Welgus HG: Neutral proteinases of human mononuclear phagocytes. Cellular differentiation markedly alters cell phenotype for serine proteinases, metalloproteinases, and tissue inhibitor of metalloroteinases. J Immunol 1991, 146: 1286-1293

Cappello M, Keshav S, Prince C, Jewell DP, Gordon S: Detection of mRNAs for macrophage products in inflammatory bowel disease by in situ hybridization. Gut 1992, 33: 1214-1219

Cattoretti F, Becker MHG, Key F, Duchrow M, Schluter C, Falle J, Ferdes J: Monoclonal antibodies against recombinant pars of the Ki-67 antigen (Mib1 and Mib3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. J Pathol 1992, 168- 357-363

Cavani A, Zambruno G, Marconi A, Manca V, Marchetti M, Giannetti A: Distinctive integrin expression in the newly forming epidermis during wound healing in humans. J Invest Dermatol 1993, 101: 600-604

Cawston TE, Billington C: Metalloproteinases in the rheumatic diseases. J Pathol 1996, 180: 115-117

Ceilley RI, Rinek MA, Zuehlke RL: Pinch grafting for chronic ulcers on lower extremities. J Dermatol Surg Oncol 3: 303-309, 1977

Chandler S, Cossins J, Lury J and Wells G: Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-α fusion protein. Biochem Biophys Res Comm 1996, 228: 421-429

Chandler S, Miller KM, Clements JM, Lury J, Corkill D, Anthony DCC, Adams SE, Gearing AJH: Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: An overview. J Neuroimmunol 72: 155-161, 1997

Chiquet-Ehrismann R, Kalla P, Pearsons CA: Participation of tenascin and transforming growth factor-β in reciprocal epithelial-mesenchymal interactions of MCF7 cells and fibroblasts. Cancer Res 1989, 49: 4322-4325

Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidium-thiocyanate-phenol-choloroform extraction. Anal Biochem 1987, 162: 156-159

Chung-Shien Lu P, Ye H, Maeda M, Azar DT: Immunolocalization and gene expression of matrilysin during corneal wound healing. Invest Ophtalmol Vis Sci 1999, 40: 20-27

Clark RAF, Lanigan JM, DellaPelle P, Manseau E, Dvorak HF, Colvin RB: Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization. J Invest Dermatol, 1982, 79: 264-269

Clark RAF: Wound repair. Overview and general considerations. In The molecular and cellular biology of wound repair. Ed. Clark RAF Plenum Press, New York 1995, pp 3-50

Cole AA, Chubinska S, Schumacher B, Huch K, Cs-Szabo G, Yao J, Mikecz K, Hasty KA, Kuettner KE: Chondrocyte matrix metalloproteinase-8. J Biol Chem 1996, 271: 11023-11026

Colige AC, Lambert CA, Nusgens BV, Lapière CM: Effect of cell-cell and cell-matrix interactions on the response of fibroblasts to epidermal growth factor *in vitro*. Biochem J 1992, 285: 215-221

Conca W, Willmroth F: Human T-lymphocytes express a member of the matrix metalloproteinase gene family. Arthritis Rheum 1994, 6: 951-956

Cossins J, Dudgeon TJ, Catlin G, Gearing AJH, Clements JM: Identification of MMP-18, a putative novel human matrix metalloproteinase. Biochem Biophys Res Comm 1996, 228: 494-498

Dagher FJ, Alongi SV, Smith A: Bacterial studies of leg ulcers. Angiology 1978, 29: 641-653
DeClerk YA, Darvill MI, Eeckhout Y, Rousseau GG: Characterization of the promoter of the gene (TIMP) encoding human tissue inhibitor of metalloproteinases-2 (TIMP-2). Gene 1994, 139: 185-191

Di Colandrea T, Wang L, Wille J, D´Armiento J, Chada KK: Epidermal expression of collagenase delays wound-healing in transgenic mice. J Invest Dermatol 1998, 111: 1029-1033

Docherty AJP, Lyons A, Smith BJ, Wright Em, Stephens PE, Harris BJ: Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity. Nature 1985, 318: 66-69

Dong Z, Kumar R, Yang X, Fidler I: Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. Cell 1997, 88: 801-810

D´Ortho M-P, Will H, Atkinson S, Butler G, Messent A, Gavrilovic J, Smith B, Timpl R, Zardi L, Murphy G: Membrane-type matrix metalloproteinases 1 and 2 (MT1-MMP and MT2-MMP) exhibit a broad spectrum proteolytic capacity comparable to many matrix metalloproteinases. Eur J Biochem 1997, 1354: 159-170

Dunsmore SE, Saarialho-Kere UK, Roby JD, Wilson CL, Matrisian LM, Welgus HG: Matrilysin expression and function in airway epithelium. J Clin Invest 1998, 102: 1321-1331

Eady RAJ, Leigh IM, Pope FM: Epidermis. In: Textbook of dermatology. Eds. Champion RH, Burton JL, Burns PA, Breathnach SM. Blackwell Science ltd, United Kingdom 1998 pp. 43-59

Eckert RL: Structure, function and differentiation of the keratinocyte. Physiol Rev 1989, 69: 1316-1346

Edwards DR, Murphy G, Reynolds JJ, Whitham SE, Docherty AJP, Angel P, Heath JK: Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. EMBO J 1987, 6: 1899-1904

Edwards DR, Beaudry PP, Laing TD, Kowal V, Leco KJ, Leco PA, Lim MS: The roles of tissue inhibitors of metalloproteinases in tissue remodeling and cell growth. Int J Obesity 1996, 20: Suppl. 3: 9-15

Falanga V, Grinnell F, Gilchrest B, Maddox YT, Moshell A: Workshop on the pathogenesis of chronic wounds. J Invest Dermatol 1994, 102: 125-127

Fariss RN, Apte SS, Olsen BR, Iwata K and Milam AH: Tissue inhibitor of metalloproteinase-3 is a component of Bruch`s membrane of the eye. Am J Pathol 1997, 150: 323-328

Ferguson MWJ, Leigh IM: Wound healing. In: Textbook of dermatology. Eds. Champion RH, Burton JL, Burns PA, Breathnach SM. Blackwell Science ltd, United Kingdom, 1998, pp 335-356

Fini ME, Parks WC, Rinehart WB, Girard MT, Matsubara M, Cook JR, West-Mays JA, Sadow PM, Burgeson RE, Jeffrey JJ, Raizman MB, Krueger RR and Zieske JD: Role of matrix metalloproteinases to re-epithelialize after corneal injury. Am J Pathol 1996, 149: 1287-1302

Fiocchi C: Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology 1998, 115: 182-205

Foidart JM, Bere EW, Yaar M Jr, Rennard SI, Gullino M, Martin GR, Katz SI: Distribution and immunoelectron microscopic localization of laminin, a noncollagenous basement membrane glycoprotein. Lab Invest 1980, 42: 336-342

Forsberg E, Hirsch E, Frohlich L, Meyer M, Ekblom P, Aszodi A, Werner S, Fassler R: Skin wounds and severed nerves heal normally in mice lacking tenascin-C. Proc Natl Acad Sci USA 1996, 93: 6594-6599

Frank S, Hübner G, Breier G, Longaker MT, Greenhalg DG, Werner S: Regulation of vascular endothelial gowth factor expression in cultured human keratinocytes. J Biol Chem 1995, 270: 12607-12613

Freije JM, Diez Itza I, Balbin M, Sanchez LM, Blasco R, Tolivia J, López-Otín C: Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. J Biol Chem 1994, 269: 16766-16773

Gailit J, Clark RAF: Wound repair in the context of extracellular matrix. Curr Opin Cell Biol 1994, 6: 717-725

Gailit J, Clark RAF: Studies in vitro on the role of α_v and β_1 integrins in the adhesion of human dermal fibroblasts to provisional matrix proteins fibronectin, vitronectin, and fibrinogen. J Invest Dermatol 1996, 106: 102-108

Gasson JC, Golde DW, Kaufman SE, Westbrook CA, Hewick RM, Kaufman RJ, Wong GG, Temple PA, Leary AC, Brown EL, Orr EC, Clark SC: Molecular characterization and expression of the gene encoding human erythroid-potentiating activity. Nature 1985, 315: 768-771

Gearing AJH, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, Drummond AH, Galloway WA, Gilbert R, Gordon JL, Leber TM, Mangan M, Miller K, Nayee P, Owen K, Patel S, Thomas W, Wells G, Wood LM, Woolley K: Processing of tumour necrosis factor-α precursor by metalloproteinases. Nature 1994, 370: 555-557

Giancotti FG, Ruoslahti E: Integrin signaling. Science 1999, 285: 1028-1032

Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V: Induction of cell migration by matrix metalloproteinase-2 cleavage of laminin-5. Science 1997, 277: 225-228

Giudice GJ, Emery DJ, Diaz LA: Cloning and primary structural analysis of the bullous pemphigoid autoantigen BP 180. J Invest Dermatol 1992, 99: 243-250

Goldberg GI, Wilhelm SM, Kronberger A, Bauer EA, Grant GA, Aisen AZ: Human fibroblast collagenase: Complete primary structure and homology to an oncogene transformation-induced rat protein. J Biol Chem 1986, 261: 6600-6605

Goldberg GI, Marmer BL, Grant GA, Eisen AZ, Wilhelm S, He C: Human 72-kilodalton type IV collagenase forms a complex with tissue inhibitor of metalloproteinases designated TIMP-2. Proc Natl Acad Sci USA 1989, 86: 8207-8211

Gomez DE, Alonso DF, Yoshij H, Thorgeirsson UP: Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. Eur J Cell Biol 1997, 74: 111-122

Graham MF, Blomqvist P, Zederfeldt B: The alimentary canal. In Wound healing. Biochemical & clinical factors. Eds. Cohen IK, Diegelmann RF, Lindblad WJ. W.B. Saunders Company, Philadelphia1992, pp 433-449

Grant DS, Leblond CP: Immunogold quantitation of laminin, type IV collagen, and heparan sulfate proteoglycan in a variety of basement membranes. J Histochem Cytochem 1988, 36: 271-283

Greene J, Wang M, Liu YE,Raymond LA, Rosen C, Shi YE: Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4. J Biol Chem 1996, 271: 30375-30380
Grinnell F, Ho CH, Wysocki A: Degradation of fibronectin and vitronectin in chronic wound fluid: analysis by cell blotting, immunoblotting, and cell adhesion assays. J Invest Dermatol 1992, 98: 410-416

Grinnell F, Zhu M: Fibronectin degradation in chronic wounds depends on the relative levels of elastase, α1-proteinase inhibitor, and α2-macroglobulin. J Invest Dermatol 1996, 106: 335-341

Grinnell F, Zhu M, Parks WC: Collagenase-1 complexes with α2-macroglobulin in the acute and chronic wound environments. J Invest Dermatol 1998, 110: 771-776

Gronski TJ, Martin RL, Kobayashi DK, Walsh BC, Holman MC, Huber M, Van Wart HE, Shapiro SD: Hydrolysis of a broad spectrum of extracellular matrix proteins by human macrophage elastase. J Biol Chem 1997, 272: 12189-12194

Gross J, Lapiere CM: Collagenolytic activity in amphibian tissues: A tissue culture assay. Physiol 1962, 48: 1014-1022

Grøndal-Hansen J, Lund LR, Ralfkiær E, Ottevanger V, Danø K: Urokinase and tissue-type plasminogen activators in keratinocytes during wound reepithelialization *in vivo*. J Invest Dermatol 1988, 90: 790-795

Grøndal-Hansen J, Ralfkiær E, Kirkeby LT, Kristensen P, Lund LR, Danø K: Localization of urokinase-type plasminogen activator in stromal cells in adenocarcinomas of the colon in humans. Am J Pathol 1991, 138: 111-117

Gururajan R, Grenet J, Lahti JM, Kidd VJ: Isolation and characterization of two novel metalloproteinase genes linked to the Cdc2L locus on human chromosome 1p36.3. Genomics 1998, 52: 101-106

Haapasalmi K, Zhang K, Tonnesen M, Olerud J, Sheppard D, Salo T, Kramer R, Cark RAF, Uitto V-J, Larjava H: Keratinocytes in human wounds express $\alpha_v\beta_6$ integrin. J Invest Dermatol 1996, 106: 42-48

Halpert I, Sires UI, Roby JD, Potter-Perigo S, Wight TN, Shapiro SD, Welgus HG, Wickline SA, Parks WC: Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. Proc Natl Acad Sci USA 1996, 93: 9748-9753

Hanemaaijer R, Sorsa T, Konttinen YT, Ding Y, Kylmäniemi M, Visser H, Hinsbergh VWM, Helaakoski T, Kainulainen T, Virkkunen J, Röntä H, Tschesche H, Salo T: Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by TNF-α and doxycycline. J Biol Chem 1997, 272: 31504-31509

Hasty KA, Jeffrey JJ, Hibbs MS, Welgus HG: The collagen substrate specificity of human neutrophil collagenase. J Biol Chem 1987, 262: 10048-10052

Hautamäki RD, Kobayashi DK, Senior RM, Shapiro SD: Requirement for macrophage elastase to cigarette-smoke-induced emphysema in mice. Science 1997, 277: 2002-2004

Hayakawa T, Yamashita K, Tanzawa K, Uchijima E, Iwata K: Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells. FEBS lett 1992, 298: 29-32

Hayakawa T, Yamashita K, Ohuchi E, Shinagawa A: Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (TIMP-2). J Cell Science 1994, 107: 2373-2379

He Cs, Wilhelm SM, Pentland AP, Marmer BL, Grant GA, Eisen AZ, Goldberg GI: Tissue cooperation in a proteolytic cascade activating human interstitial collagenase. Proc Natl Acad Sci USA 1989, 86: 2632-2636

Heppner KJ, Matrisian LM, Jensen RA, Rodgers WH: Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. Am J Pathol 1996, 149:273-282

Herouy Y, May AE, Pornschlegel G, Grenz H, Preissner KT, Schöpf E, Norgauer J, Vanscheidt W: Lipodermatosclerosis is characterized by elevated expression and activation of matrix metalloproteinases: Implications for venous ulcer formation. J Invest Dermatol 1998, 111: 822-827

Herrick SE, Sloan P, McGurk M, Freak L, McCollum CN, Ferguson MWJ: Sequential changes in histologic pattern and extracellular matrix deposition during the healing of chronic venous ulcers. Am J Pathol 1992, 141: 1085-1095

Highley HR, Ksander GA, Gerhardt CO, Falanga V: Extravasation of macromolecules and possible trapping of transforming growth factor-β in venous ulceration. Br J Dermatol 1995, 132: 79-85

Holmbeck K, Bianco P, Caterina C, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, Ward JM, Birkedal-Hansen H: MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadeguate collagen turnover. Cell 1999, 99: 81-92

Howard EW, Bullen EC, Banda MJ: Preferential inhibition of 72- and 92-kDa gelatinases by tissue inhibitor of metalloproteinases-2. J Biol Chem 1991, 266: 13070-13075

Hugot J-P, Zouali H, Lesage S, Thomas G: Etiology of the inflammatory bowel diseases. Int J Colorect Dis 1999, 14: 2-9

Huhtala P, Humphries MJ, McCarthy JB, Tremble PM, Werb Z, Damsky CH: Cooperative signaling by α5β1 and α4β1 integrins regulates metalloproteinase gene expression in fibroblasts adhering to fibronectin. J Cell Biol 1995, 129: 867-879

Hynes RO: Integrins: Versatility, modulation, and signaling in cell adhesion. Cell 1992, 69: 11-25

Imper V, van Wart HE: Matrix metalloproteinase substrate recognition. In Matrix metalloproteinases. Eds. Parks WC, Mecham RP. Academic Press, California, 1998, pp 219-242

Inoue M, Kratz G, Haegerstrand A, Ståhle-Bäckdahl M: Collagenase expression is rapidly induced in wound-edge keratinocytes after acute injury in human skin, persists during healing, and stops at re-epithelialization. J Invest Dermatol 1995, 104: 479-483

Ito A, Sato T, Iga T, Mori Y: Tumor necrosis factor bifunctionally regulates matrix metalloproteinases and tissue inhibitor of metalloproteinases (TIMP) production by human fibroblasts. FEBS Lett 1990, 269: 93-95

Itoh T, Ikeda T, Gomi H, Nakao S, Suzuki T, Itohara S: Unaltered secretion of β-amyloid precursos protein in gelatinase A (MMP-2) deficient mice. J Biol Chem 1997, 272: 22389-22392

Itoh T, Tanioka M, Yoshida T, Nishimoto H, Itohara S: Reduced angiogenesis and tumor progression in gelatinase A deficient mice. Cancer Res 1998, 58: 1048-1051

James TW, Wagner R, White LA, Zwolak RM, Brinckerhoff CE: Induction of collagenase and stromelysin gene expression by mechanical injury in a vascular smooth muscle-derived cell line. J Cell Physiol 1993, 157: 426-437

Johansson N, Westermarck J, Leppä S, Häkkinen L, Koivisto L, López-Otin C, Peltonen J, Heino J, Kähäri V-M: Collagenase-3 (matrix metalloproteinase-13) gene expression by HaCaT keratinocytes is enhanced by tumor-necrosis factor-α and transforming growth factor-β. Cell Growth Differ 1997a, 8: 243-250

Johansson N, Saarialho-Kere UK, Airola K, Herva R, Nissinen L, Westermarck J, Vuorio E, Heino J, Kähäri VM: Collagenase-3 (MMP-13) is expressed by hypertrophic chondrocytes, periosteal cells, and osteoblasts during fetal bone development. Dev Dyn 1997b, 208: 387-397

Johansson N, Airola K, Grénman R, Kariniemi A-L, Saarialho-Kere UK, Kähäri V-M: Expression of collagenase-3 (matrix metalloproteinase-13) in squamous cell carcinomas of the head and neck. Am J Pathol 1997c, 151: 499-508

Johansson N, Vaalamo M, Grénman S, Hietanen S, Klemi P, Saarialho-Kere U, Kähäri V-M: Collagenase-3 (MMP-13) is expressed by tumor cells in invasive vulvar squamous cell carcinomas. Am J Pathol 1999, 154: 469-480

Johnatty RN, Taub D, Reeder SP, Turcovski-Corrales SM; Cottam DW, Stephenson TJ, Rees RC: Cytokine and chemokine regulation of proMMP-9 and TIMP-1 production by human peripheral blood lymphocytes. J Immunol 1997, 158: 2327-2333

Johnson MD, Kim H-RC, Chesler L, Tsao-Wu G, Bouck N, Polverini P: Inhibition of angiogenesis by tissue inhibitor of metalloproteinase. J Cell Physiol 1994, 160: 194-202

Jones JL, Walker RA: Control of matrix metalloproteinase activity in cancer. J Pathol 1997, 183: 377-379

Jones MK, Tomikawa M, Mohajer B, Tarnawski AS: Gastrointestinal mucosal regeneration: role of growth factors. Front Biosci 1999, 4: 303-309

Juhasz I, Murphy GF, Yan H-C, Herlyn M, Albelda SM: Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing in vivo. Am J Pathol 1993, 143: 1458-1469

Kariniemi A-L, Kauppinen K: Ihon rakenne ja toiminta. In Ihotautioppi. Eds Kariniemi A-L, Kauppinen K. WSOY, Porvoo 1989, p 11

Keski-Oja J, Lohi J, Tuuttila A, Tryggvason K, Vartio T: Proteolytic processing of the 72,000 Da type IV collagenase by urokinase plasminogen activator. Exp Cell Res 1992, 202: 471-476

Kiistala U, Mustakallio KK: Dermo-epidermal separation with suction. Electron microscopic and histochemical study of initial events of blistering on human skin. J Invest Dermatol 1968, 100: 335-342

Kim JP, Chen JD, Woodley DT: Mechanism of human keratinocyte migration on fibronectin: Unique roles of RGD site and integrins. J Cell Physiol 1992, 151: 443-450

Kim JP, Chang K, Chen JD, Kramer RH, Woodley DT: Vitronectin-driven human keratinocyte locomotion is mediated by the $\alpha_v\beta_5$ integrin receptor. J Biol Chem 1994, 43: 26926-26932

Knox JB, Sukhova GK, Whittemore AD, Libby P: Evidence for altered balance between matrix metalloproteinases and their inhibitors in human aortic diseases. Circulation 1997, 95: 205-212

Knäuper V, López-Otin C, Smith B, Knight G, Murphy G: Biochemical characterization of human collagenase-3. J Biol Chem 1996, 271: 10917-10923
Knäuper V, Smith B, López-Otin C, Murphy G: Activation of progelatinase B (proMMP-9) by active collagenase-3 (MMP-13). Eur J Biochem 1997, 248: 369-373

Knäuper V, Murphy G: Membrane type matrix metalloproteinases and cell surface-associated activation of matrix metalloproteinases. In Matrix metalloproteinases. Eds. Parks WC, Mecham RP. Academic Press, California 1998, pp199-218

Kohga S, Harvey SR, Weaver RM, Markus G: Localization of plasminogen activators in human colon cancer by immunoperoxidase staining. Cancer Res 1985, 45: 1787-1796

Konturek JW, Brzozowski T, Konturek SJ: Epidermal growth factor in protection, repair, and healing of gastroduodenal mucosa. J Clin Gastroenterol. 1991, 13(Suppl 1): 88-97

Konturek SJ, Brzozowski T, Majka j, Dembinski A, Slomiany A, Slomiany BL: Transforming growth factor-alpha and epidermal growth factor in protection and healing of gastric mucosal injury. Gastroenterol 1992, 27: 649-655

Kyriakides TR, Tam JW, Bornstein P: Accelerated wound healing in mice with a disruption of the thrombospondin 2 gene. J Invest Dermatol 1999, 113: 782-787

Kähäri V-M, Saarialho-Kere UK: Matrix metalloproteinases in skin. Exp Dermatol 1997, 6: 199-213

Kähäri V-M, Saarialho-Kere UK: Matrix metalloproteinases and their inhibitors in tumour growth and invasion. Ann Med 1999, 31: 34-45

Lacy ER: Epithelial restitution in the gastrointestinal tract. J Clin Gastroenterol 1988, 10 (Suppl. 1): 72-77

Larjava H, Salo T, Haapasalmi K, Kramer RH, Heino J: Expression of integrins and basement membrane components by wound keratinocytes. J Clin Invest 1993a: 1425-1435

Larjava H, Lyons G, Salo T, Mäkelä M, Koivisto L, Birkedal-Hansen H, Akiyama SK, Yamada KM, Heino J: Anti-integrin antibodies induce type IV collagenase expression in keratinocytes. J Cell Physiol 1993b, 157: 190-200

Latjinhouwers MA, Bergers M, van Bergen BH, Sprujit KI, Andriessen MP, Schalkwijk J: Tenascin expression during wound healing in human skin. J Pathol 1996, 178: 30-35

Leco KJ, Khokha R, Pavloff N, Hawkes S, Edwards DR: Tissue inhibitor of metalloproteinases-3 (TIMP-3) is an extracellular matrix -associated protein with a distinctive pattern of expression in mouse cells and tissues. J Biol Chem 1994, 269: 9352-9360

Leco KJ, Apte SS, Taniguchi GT, Hawkes SP, Khokha R, Schultz GA, Edwards DR: Murine tissue inhibitor of metalloproteinases-4 (TIMP-4): cDNA isolation and expression in adult mouse tissues. FEBS Lett 1997, 401: 213-217

Lees M, Taylor DJ, Woolley DE: Mast cell proteinases activate precursor forms of collagenase and stromelysin, but not of gelatinases A and B. Eur J Biochem, 1994: 223: 171-177

Leivo I, Tani T, Laitinen L, Bruns R, Kivilaakso E, Lehto V-P, Burgeson RE, Virtanen I: Anchoring complex components laminin-5 and type VII collagen in intestine: Association with migrating and differentiating enterocytes. J Histochem Cytochem 1996, 44: 1267-1277

Lepistö J, Peltonen J, Vähä-Kreula M, Niinikoski J, Laato M: Platelet-derived growth factor isoforms PDGF-AA, -AB and -BB exert specific effects on collagen gene expression and mitotic activity of cultured human wound fibroblasts. Biochem Biophys Res Comm 1995, 209: 393-399

Lightner VA: Tenascin: Does it play a role in epidermal morphogenesis and homeostasis? J Invest Dermatol 1994, 102: 273-277

Ligumsky M, Simon PL, Karmeli F, Rachmilewitz D: Role of interleukin 1 in inflammatory bowel disease – enhanced production during active disease. Gut 1990, 31: 686-689

Liu Ye, Wang M, Greene J, Su J, Ullrich S, Li H, Sheng S, Alexander P, Sang QA, Shi YE: Preparation and characterization of recombinant tissue inhibitor of metalloproteinases (TIMP-4). J Biol Chem 1997, 272: 20479-20483

Liu Z, Shipley JM, Vu T, Zhou X, Diaz LA, Werb Z, Senior RM: Gelatinase B-deficient mice are resistant to bullous pemphigoid. J Exp Med 1998, 188: 475-482

Llano E, Pendas AM, Knäuper V, Sorsa T, Salo T, Salido E, Murphy G, Simmer JP, Bartlett JD, López-Otín C: Identification and structural and functional characterization of human enamelysin (MMP-20). Biochemistry 1997, 36: 15101-15108

Llano E, Pendas AM, Freije PJ, Nakano A, Knäuper V, Murphy G, López-Otin C: Identification and characterization of human MT5-MMP, a new membrane-bound activator of progelatinase A overexpressed in brain tumors. Cancer Res 1999, 59: 2570-2576

Lohi J, Leivo I, Tani T, Kiviluoto T, Kivilaakso E, Burgeson RE, Virtanen I: Laminins, tenascin and type VII collagen in colorectal mucosa. Histochem J 1996, 28: 431-440

Loots MAM, Lamme EN, Zeegelaar J, Mekkes J, Bos JD, Middelkoop E: Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers *versus* acute wound. J Invest Dermatol 1998, 111: 850-857

Lund LR, Rømer J, Bugge TH, Nielsen BS, Frandsen TL, Degen JL, Stephens RW, Danø K: Functional overlap between two classes of matrix-degrading proteases in wound healing. EMBO J 1999, 18: 4645-4656

Madlener M, Mauch C, Conca W, Brauchle M, Parks WC, Werner S: Regulation of the expression of stromelysin-2 by growth factors in keratinocytes: implications for normal and impaired wound healing. Biochem J 1996, 320: 659-664

Madlener M, Parks WC, Werner S: Matrix metalloproteinases (MMPs) and their physiological inhibitors (TIMPs) are differentially expressed during excisional skin wound repair. Exp Cell Res 1998, 242: 201-210

Martens MFWC, Huyben CMLC, Hendriks TH: Collagen synthesis in fibroblasts from human colon: regulatory aspects and differences with skin fibroblasts. Gut 1992, 33: 1664-1670

Martin DC, Fowlkes JL, Babic B, Khokha R: Insulin-like growth factor II signaling in neoplastic proliferation is blocked by transgenic expression of the metalloproteinase inhibitor TIMP-1. J Cell Biol 1999, 146: 881-892

Mast B: Healing in other tissues. Surg Clin North America 1997, 77: 529-547

Mauch C, Adelmann-Grill B, Hatamochi A, Krieg T: Collagenase gene expression in fibroblasts is regulated by a three-dimensional contact with collagen. FEBS Lett 1989, 250: 301-305

Mauch C, Krieg T, Bauer EA: Role of the extracellular matrix in the degradation of connective tissue. Arch Dermatol Res 1994, 287: 107-114

Mauviel A, Chung KY, Agarwal A, Tamai K, Uitto J: Cell specific induction of distinct oncogenes of the jun family is responsible for differential regulation of collagenase gene expression by transforming growth factor-beta in fibroblasts and keratinocytes. J Biol Chem 1996, 271: 10917-10923

McClain SA, Simon M, Jones E, Nandi A, Gailit JO, Tonnesen MG, Newman D, Clark RAF: Mesenchymal cell activation is the rate-limiting step of granulation tissue induction. Am J Pathol 1996, 149: 1257-1270

McDonald JA, Broekelmann TJ, Matheke ML, Crouch E, Koo M, Kuhn C III: A monoclonal antibody to the carboxyterminal domain of procollagen type I visualizes collagen synthesizing fibroblasts. J Clin Invest 1986, 78: 1237-1244

McDonnel S, Navre M, Coffey RJ, Matrisian L: Expression and localization of the matrix-metalloproteinase pump-1 (MMP-7) in human gastric and colon carcinomas. Mol Carcinogen 1991, 4: 527-533

McLean WHI, Pulkkinen L, Smith FJD, Rugg EL, Lane EB, Bullrich F, Burgeson RE, Amano S, Hudson DL, Owaribe K, McGrath JA, McMillan JR, Eady RA, Leigh IM, Christiano AM, Uitto J: Loss of plectin causes epidermolysis bullosa with muscular dystrophy: cDNA cloning and genomic organization. Genes Dev 1996, 10: 1724-1735

Mecham RP, Heuser JE: The elastic fiber. In Cell biology of extracellular matrix. Ed. Elisabeth D. Hay. Plenum Press, New York 1991, pp 79-110

Mekkes JR, Zeegelaar JE, Westerhof W: Quantitative and objective evaluation of wound debriding properties of collagenase and fibrinolysin/deoxyribonuclease in a necrotic ulcer animal model. Arch Dermatol Res 1998, 290: 152-157

Messent AJ, Tuckwell DS, Knäuper V, Humphries MJ, Murphy G: Effects of collagenase-cleavage of type I collagen on $\alpha 2\beta 1$ integrin-mediated cell adhesion. J Cell Sci 1998, 111: 1127-1135

Mignatti P, Rifkin DB: Biology and biochemistry of proteinases in tumor invasion. Phys Rev 1993, 73: 161-195

Mitchell PG, Magna HA, Reeves LM, Lopresti-Morrow LL, Yocum SA, Rosner PJ, Geoghegan KF, Hambor JE: Cloning, expression and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. J Clin Invest 1996, 97: 761-768

Montesano R, Orci L: Transforming growth factor β stimulates collagen-matrix contraction by fibroblasts: implications for wound healing. Proc Natl Acad Sci USA 1988, 85: 4894-4897

Moore K, Ruge F, Harding KG: T lymphocytes and the lack of activated macrophages in wound margin biopsies from chronic leg ulcers. Br J Dermatol 1997, 137: 188-194

Mudgett JS, Hutchinson NI, Chartrain NA, Forsyth AJ, McDonnel J, Singer II, Bayne EK, Flanagan J, Kawka D, Shen CF, Stevens K, Chen H, Trumbauer M, Visco DM: Susceptibility of stromelysin-1 deficient mice to collagen-induced arthritis and cartilage destruction. Arthritis Rheum 1998, 41: 110-121

Murch SH, Braegger CP, Walker-Smith JA, MacDonald TT: Location of tumour necrosis factor α by immunohistochemistry in chronic inflammatory bowel disease. Gut 1993, 34:1705-1709

Murphy G, Cockett MI, Ward RV, Docherty AJP: Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan: a quantitative comparison of activities of 95 kDa and 75 kDa gelatinases, stromelysin-1 and -2 and punctuated metalloproteinase (PUMP). Biochem J 1991, 277: 277-279

Murphy G, Allan JA, Willenbrock F, Cockett MI, O`Connel PO, Docherty AJP: The role of the C-terminal domain in collagenase and stromelysin specificity. J Biol Chem 1992, 267: 9612-9618

Murphy G, Stanton H, Cowell S, Butler G, Knäuper V, Atkinson S, Gavrilovic J: Mechanisms for pro matrix metalloproteinase activation. APMIS 1999, 107: 38-44

Mustoe TA, Landes A, Cromack DT, Mistry D, Griffin A, Deuel TJ, Pierce GF: Differential acceleration of healing of surgical incisions in the rabbit gastrointestinal tract by platelet-derived growth factor and transforming growth factor, type beta. Surgery 1990, 108: 324-330

Mäkelä M, Larjava H, Pirilä E, Maisi P, Salo T, Sorsa T, Uitto V-J: Matrix metalloproteinase-2 (gelatinase A) is related to migration of keratinocytes. Exp Cell Res 1999, 251: 67-78

Nagase H: Stromelysins 1 and 2. In Matrix metalloproteinases. Eds. Parks WC, Mecham RP. Academic Press, California 1998, pp. 43-84

Nakamura H, Fujii Y, Ohuchi E, Yamamoto E, Okada Y: Activation of the precursor of stromelysin-2 and its interactions with other matrix metalloproteinases. Eur J Biochem 1998, 253: 67-75

Nelzen O, Bergqvist D, Lindhagen A: The prevalence of chronic lower-limb ulceration has been underestimated: results of a validated population questionnaire. Br J Surg 1996, 83: 255-258

Nemeth JA, Goolsby CL: TIMP-2, a growth-stimulatory protein from SV-40-transformed human fibroblasts. Exp Cell Res 1993, 207: 376-382

Newell KJ, Witty JF, Rodgers WH, Matrision LM: Expression and localization of matrix degrading metallproteinases during colorectal tumorigenesis. Mol Carcinogen 1994, 10: 199-206

Nicosia RF, Tuszynski GP: Matrix-bound thrombospondin promotes angiogenesis in vitro. J Cell Biol 1994, 124: 183-193

Niessen CM, Hogervorst F, Jaspars LH, de Melker AA, Delwel Go, Hulsman EH, Kuikman I, Sonnenberg A: The alpha 6 beta 4 integrin is a receptor for both laminin and kalinin. Exp Cell Res 1994, 211: 360-367

Nomura H, Sato H, Seiki M, Mai M, Yasunori O: Expression of membrane-type matrix metalloproteinase in human gastric carcinomas. Cancer Res 1995, 55: 3263-3266

Nwomeh BC, Liang H-X, Cohen IK, Yager DR: MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. J Surg Res 1999, 81: 189-195

O`Connor CM, FitzGerald MX: Matrix metalloproteinases and lung disease. Thorax 1994, 49: 602-609

Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y: Membrane type 1 metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. J Biol Chem 1997, 272: 2446-2451

Ohta S, Imai K, Yamashita K, Matsumoto T, Azumono I, Okada Y: Expression of matrix metalloproteinase 7 (matrilysin) in human osteoarthritic cartilage. Lab Invest 1998, 78: 79-87

Oikarinen A, Kylmäniemi M, Autio-Harmainen H, Autio P, Salo T: Demontration of 72-kDa and 92-kDa forms of type IV collagenase in human skin: Variable expression in various blistering diseases, induction during re-epithelialization, and decrease by topical glucocorticoids. J Invest Dermatol 1993, 101: 205-210

Okada A, Bellocq J-P, Rouyer N, Chenard M-P, Rio M-C, Chambon P, Basset P: Membrane-type matrix metalloproteinase (MT-MMP) gene is expressed in stromal cells of human colon, breast, and head and neck carcinomas. Proc Natl Acad Sci USA 1995, 92: 2730-2734

Okada A, Tomasetto C, Lutz Y, Bellocq J-P, Rie M-C, Basset B: Expression of matrix metalloproteinases during rat skin wound healing: Evidence that membrane-type-I metalloproteinase is a stromal activator of pro-gelatinase A. J Cell Biol 1997, 137: 67-77

Okumura Y, Sato H, Seiki M, Kido H: Proteolytic activation of membrane type 1 matrix metalloproteinase by human plasmin. A possible cell surface activator. FEBS Lett 1997, 402: 181-184

Orian-Rousseau V, Aberdam D, Fontao L, Chevalier L, Meneguzzi G, Kedinger M, Simon-Assmann P: Developmental expression of laminin-5 and HD1 in the intestine: epithelial to mesenchymal shift for the laminin gamma-2 chain subunit deposition. Dev Dyn 1996, 206: 12-23

O´Toole EA, Marinkovich MP, Hoeffler WK, Furthmayr H, Woodley DT: Laminin-5 inhibits human keratinocyte migration. Exp Cell Res 1997, 233: 330-339

Palolahti M, Lauharanta J, Stephens RW, Kuusela P, Vaheri A: Proteolytic activity in leg ulcer exudate. Exp Dermatol 1993, 2: 29-37

Pallone F, Fais S, Squarcia O, Biancone L, Pozzilli P, Boirivant M: Activation of peripheral blood and intestinal lamina propria lymphocytes in Crohn`s disease. In vivo state of activation and in vitro response to stimulation as defined by the expression of early activation antigens. Gut 1987, 28: 745-753

Pilcher BK, Sudbeck BD, Dumin JA, Welgus HG, Parks WC: Collagenase-1 and collagen in epidermal repair. Arch Dermatol Res 1998, 290 (Suppl): 37-46

Patterson BD, Sang QXA: Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). J Biol Chem 1997, 272: 28823-28825

Pei D, Majmudar G, Weiss SJ: Hydrolytic inactivation of a breast carcinoma cell-derived serpin by human stromelysin-3. J Biol Chem 1994, 269: 25849-25855

Pei D, Weiss SJ: Furin-dependent intracellular activation of the human stromelysin-3 zymogen. Nature 1995, 375: 244-247

Pei D, Weiss SJ: Transmembrane-deletion mutants of the membrane-type matrix metalloproteinse-1 process progelatinase A and express intrinsic matrix-degrading activity. J Biol Chem 1996, 271: 9135-9140

Pei D: Identification and characterization of the fifth membrane-type matrix metalloproteinase MT5-MMP. J Biol Chem 1999, 274: 8925-8932

Peltonen J, Larjava H, Jaakkola S, Gralnick H, Akiyama SK, Yamada SS, Yamada KM, Uitto J: Localization of integrin receptors for fibronectin, collagen, and laminin in human skin. Variable expression in basal and squamous cell carcinomas. J Clin Invest 1989, 84: 1916-1923

Pendás AM, Knäuper V, Puente XS, Llano E, Mattei M-G, Apte S, Murphy G, López-Otin C: Identification and characterization of a novel human matrix metalloproteinase with unique structural characteristics, chromosomal location, and tissue distribution. J Biol Chem 1997, 272: 4281-4286

Pender SLF, Lionetti P, Murch SH, Wathan N, MacDonald TT: Proteolytic degradation of intestinal mucosal extracellular matrix after lamina propria T-cell activation. Gut 1996, 39: 284-290

Pender SLF, Tickle SP, Docherty AJP, Howie D, Wathen NC, MacDonald TT: A major role for matrix metalloproteinases in T cell injury in the gut. J Immunol 1997, 158: 1582-1590

Pender SLF, Breese EJ, Günther U, Howie D, Wathen NC, Schuppan D, MacDonald TT: Suppression of T cell-mediated injury in human gut by interleukin 10: Role of matrix metalloproteinases. Gastroenterology 1998a, 115: 573-583

Pender SLF, Fell JME, Chamow SM, Ashkenazi A, MacDonald TT: A p55 TNF receptor immnoadhesin prevents T cell-mediated intestinal injury by inhibiting matrix metalloproteinase production. J Immunol 1998b, 160: 4098-4103

Petersen MJ, Woodley DT, Stricklin GP, O´Keefe EJ: Enhanced synthesis of collagenase by human keratinocytes cultured on type I or type IV collagen. J Invest Dermatol 1990, 94: 341-346

Petri JB, König S, Haupt B, Haustein U-F, Herrmann K: Molecular analysis of different phases in human wound healing. Exp Dermatol 1997, 6: 133-139

Petruzelli L, Takami M, Humes D: Structure and funtion of cell adhesion molecules. Am J Med 1999, 106: 467-476

Pilcher BK, Dumin JA, Sudbeck BD, Krane SM, Welgus HG, Parks WC: The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. J Cell Biol 1997, 137: 1445-1457

Planus E, Galiancy S, Matthay M, Laurent V, Gavrilovic J, Murphy G, Clérici C, Isabey D, Lafuma C, d`Ortho M-P: Role of collagenase in mediating in vitro alveolar epithelial wound repair. J Cell Sci 1999, 112: 243-252

Podolsky DK: Inflammatory bowel disease. New Engl J Med 1991, 325: 928-937

Polette M, Nawrocki B, Gilles C, Sato H, Seiki M, Tournier J-M, Birembaut P: MT-MMP expression and localization in human lung and breast cancers. Virchows Arch 428: 29-35, 1996

Poulsom R, Pignatelli M, Stetler-Stevenson WG, Liotta LA, Wright PA, Jeffery RE, Longcroft JM, Rogers L, Stamp GWH: Stromal expression of 72 kda type IV collagenase (MMP-2) and TIMP-2 mRNAs in colorectal neoplasia. Am J Pathol 1992, 141: 389-396

Pope FM: Dermis. in Textbook of dermatology. Eds. Champion RH, Burton JL, Burns PA, Breathnach SM. Blackwell Science ltd, United Kingdom 1998, pp. 59-92

Powe DG, Brough Jl, Carter GI, Ravery V, Angulo J, Shamsa F, et al: TIMP-3 mRNA expression is regionally increased in moderately and poorly differentiated colorectal adenocarcinoma. Br J Cancer 1997, 75: 199-206

Prockop DJ, Kivirikko K: Collagens: Molecular biology, diseases and potentials for therapy. Annu Rev Biochem 1995, 64: 403-434

Prosser IW, Stenmark KR, Suthar M, Crouch EC, Mecham RP, Parks WC: Regional heterogeneity of elastin and collagen gene expression in intralobar arteries in response to hypoxic pulmonar hypertension as demonstrared by in situ hybridization. Am J Pathol 1989, 135: 1073-1088

Puente XS, Pendas AM, Llano E, Velasco G, López-Otín C: Molecular cloning of a novel membrane-type matrix metalloproteinase from a human breast carcinoma. Cancer Res 1996, 56: 944-949

Pulford KAF, Rigney EM, Micklem KF, Stross WP, Fatter KC, Mason DY: KP1: a new monoclonal antibody that detects a monocyte/macrophage associated antigene in routinely processed tissue sections. J Clin Pathol 1989, 42: 414-421

Pyke C, Ralfkiær E, Huhtala P, Hurskainen T, Danø K, Tryggvason K: Localization of messenger RNA for M_r 72,000 and 92,000 type IV collagenases in human skin cancers by *in situ* hybridization. Cancer Res 1992, 52: 1336-1341

Pyke C, Salo S, Ralfkiær E, Rømer J, Danø K, Tryggvason K: Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. Cancer Res 1995, 55: 4132-4139

Quaglino D Jr, Nanney LB, Kennedy R, Davidson JM: Transforming growth factor-beta stimulates wound healing and modulates extracellular matrix gene expression in pig skin. Lab Invest 1990, 63: 307-319

Quaglino D Jr, Nanney LB, Ditesheim JA, Davidson JM: Transforming growth factor-beta stimulates wound healing and modulates extracellular matrix gene expression in pig skin: Incisional wound model. J invest Dermatol 1991, 97: 34-42

Raghow R: Role of transforming growth factor-beta in repair and fibrosis. Chest 1991, 99(suppl. 3): 61-65

Ranki A, Nyberg M, Ovod V, Haltia M, Elovaara I, Raininko R, Haapasalo H, Krohn K: Abundant expression of HIV Nef and Rev proteins in brain astrocytes in vivo is associated with dementia. AIDS 1995, 9: 1001-1008

Rao CN, Ladin DA, Liu YY, Chilukuri C, Hou ZZ, Woodley DT: α1-antitrypsin is degraded and non-functional in chronic wounds but intact and functional in acute wounds: the inhibitor protects fibronectin from degradation by chronic wound fluid enzymes. J Invest Dermatol 1995, 105: 572-578

Rapala K: The effect of tumor necrosis factor-alpha on wound healing. An experimental study. Ann Chir Gynaecol Suppl 1996, 211: 1-53

Rapala K, Heino J, Peltonen J, Laato M: The effects of interleukin-1 and prostaglandin E2 on accumulation of collagen and steady-state levels of pro alpha 1(I) collagen messenger RNA in experimental granulation tissue in rats. Arch Dermatol Res 1997, 289: 219-223

Ravanti L, Heino J, López-Otín C, Kähäri V-M: Induction of collagenase-3 in human skin fibroblasts by three-dimensional collagen is mediated by p38 mitogen-activated protein kinase. J Biol Chem 1999a, 274: 2446-2455

Ravanti L, Häkkinen L, Larjava H, Saarialho-Kere U, Foschi M, Han J, Kähäri V-M: Transforming growth factor-β induces collagenase-3 (MMP-13) expression by human gingival fibroblats vie p38 mitogen-activated protein kinase. J Biol Chem 1999b, in press

Reed MJ, Vernon RB, Abrass IB, Sage HE: TGF-β1 induces the expression of type I collagen and SPARC, and enhances contraction of collagen gels, by fibroblasts from young and aged donors. J Cell Physiol 1994, 158: 169-179

Reboul P, Pelletier J-P, Tardif G, Cloutier J-M, Martel-Pelletier J: The new collagenase, collagenase-3 is expressed and synthesized human chondrocytes and synoviocytes. J Clin Invest 1996, 97: 2011-2019

Riikonen T, Westermarck J, Koivisto L, Broberg A, Kähäri V-M, Heino J: Integrin α2β1 is a positive regulator of collagenase (MMP-1) and collagen α1(I) gene expression. J Biol Chem 1995, 270: 13548-13552

Robbins SL, Kumar V, Cotran RS: Wound healing. In Robbins Pathologic basis of disease. Eds. Robbins SL, Kumar V, Cotran RS. W.B. Saunders company, Philadelphia, 1989a pp. 73-86

Robbins SL, Kumar V, Cotran RS: Small intestine & Colon. In Robbins Pathologic basis of disease. Eds. Robbins SL, Kumar V, Cotran RS. W.B. Saunders company, Philadelphia, 1989b pp. 867-889

Rodgers WH, Matrisian LM, Giudice LC, Dsupin B, Cannon, Svitek C, Gorstein F, Osteen KG: Patterns of matrix metalloproteinase expression in cycling endometrium imply differential functions and regulation by steroid hormones. J Clin Invest 1994, 94: 946-953

Rousselle P, Lundstrum GP, Keene DR, Burgeson RE: Kalinin: an epithelium-specific basement membrane adhesion molecule that is a componen of anchoring filaments. J Cell Biol 1991 114: 567-576

Ross MH, Reith EJ: Small Intestine & Large Intestine. In Histology: A text and atlas. Eds. Ross MH, Reith EJ. Harper & Row Publishers, J.B. Lippincott Company, 1985a, pp. 423-440

Ross MH, Reith EJ: Connective tissue cells. In Histology: A text and atlas. Eds. Ross MH, Reith EJ. Harper & Row Publishers, J.B. Lippincott Company, 1985b, pp. 92-100

Rømer J, Lund LR, Eriksen J, Ralfkiær E, Zeheb R, Gelehrter TD, Danø K, Kristensen P: Differential expression of urokinase-type plasminogen activator and its type-1 inhibitor during healing of mouse skin wounds. J Invest Dermatol 1991, 97: 803-811

Rømer J, Lund LR, Eriksen J, Pyke C, Kristensen P, Danø K: The receptor for urokinase-type plasminogen activator is expressed by keratinocytes at the leading edge during re-epithelialization of mouse skin wounds. J Invest Dermatol 1994, 102: 519-522

Rømer J, Bugge TH, Pyke C, Lund LR, Flick MJ, Degen JL, Danø K: Impaired wound healing in mice with a disrupted plasminogen gene. Nature Medicine 1996, 2: 287-292

Saarialho-Kere UK, Chang ES, Welgus HG and Parks WC: Distinct localization of collagenase and TIMP expression in wound healing associated with ulcerative pyogenic granuloma. J Clin Invest 1992, 90: 1952-1957

Saarialho-Kere UK,Chang ES, Welgus HG and Parks WC: Expression of interstitial collagenase, 92 kDa gelatinase and TIMP-1 in granuloma annulare and necrobiosis lipoidica diabeticorum. J Invest Dermatol 1993a, 100: 335-342

Saarialho-Kere UK, Pentland AP, Olerud JE, Welgus HG, Parks WC: Cell-matrix interactions modulate interstitial collagenase expression by human keratinocytes actively involved in wound healing. J Clin Invest 1993b, 92: 2858-2866

Saarialho-Kere UK, Welgus HG, Parks WC: Divergent mechanisms regulate interstitial collagenase and 92 kDa gelatinase by endotoxin in monocytic-like cells. J Biol Chem 1993c, 268: 17354-17361

Saarialho-Kere UK, Pentland AP, Birkedal-Hansen H, Parks WC, Welgus HG: Distinct populations of basal keratinocytes express stromelysin-1 and stromelysin-2 in chronic wounds. J Clin Invest 1994, 94: 79-88

Saarialho-Kere UK, Crouch EC, Parks WC: Matrix metalloproteinase matrilysin is constitutively expressed in adult human exocrine epithelium. J Invest Dermatol 1995, 105: 190-196

Saarialho-Kere UK, Kerkelä E, Jeskanen L, Hasan T, Pierce R, Starcher B, Raudasoja R, Ranki A, Oikarinen A, Vaalamo M: Accumulation of matrilysin (MMP-7) and macrophage metalloelastase (MMP-12) in actinic damage. J Invest Dermatol, 1999, 113: 664-672

Sage EH, Bornstein P: Extracellular proteins that modulate cell-matrix interactions. J Biol Chem 1991, 266: 14831-14834

Saksela O, Rifkin DB: Cell associated plasminogen activation: Regulation and physiological functions. Ann Rev Cell Biol 1988, : 93-126

Salo T, Lyons JG, Rahemtull F, Birkedal-Hansen H, Larjava H: Transforming growth factor-β1 up-regulates type IV collagenase expression in cultured human keratinocytes. J Biol Chem 1991, 266: 11436-114411

Salo T, Mäkelä M, Kylmäniemi M, Autio-Harmainen H, Larjava H: Expression of matrix metalloproteinase-2 and -9 during early human wound healing. Lab Invest 1994, 70: 176-182

Sasaki T, Gohring W, Mann K, Maurer P, Hohenester E, Knäuper V, Murphy G, Timpl R: Limited cleavage of extracellular matrix protein BM-40 by matrix metalloproteinases increases its affinity for collagens. J Biol Chem 1997, 272: 9273-9243

Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M: A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 370: 61-65, 1994

Sato H, Kinoshita T, Takino T, Nakayama K, Seiki M: Activation of a recombinant membrane type 1-matrix metalloproteinase (MT1-MMP) by furin and its interaction with tissue inhibitor of metalloproteinases (TIMP)-2. FEBS Lett 1996, 393: 101-104

Savage FJ, Lacombe DL, Boulos PB, Hembry RM: Role of matrix metalloproteinases in healing of colonic anastomosis. Dis Colon Rectum 1997, 40: 962-70

Savage FJ, Lacombe DL, Hembry RM, Boulos PB: Effect of colonic obstruction of tne distribution of matrix metalloproteinases during anastomotic healing. Br J Surg 1998, 85: 72-75

Scharffetter K, Kulozik M, Stoltz W, Lankat-Buttgereit B, Hatamochi A, Söhnchen R, Krieg T: Localization of collagen α_1(I) gene expression during wound healing by in situ hybridization. J Invest Dermatol 1989, 93: 405-412

Schiro JA, Chan BM, Roswit WT, Kassner PD, Pentland AP, Hemler ME, Eisen AZ, Kupper TS: Integrin alpha 2 beta 1 (VLA-2) mediates reorganization and contraction of collagen matrices by human cells. Cell 1991, 67: 403-410

Schäfer BM, Maier K, Eickhoff U, Todd RF, Kramer MD: Plasminogen activation in healing human wounds. Am J Pathol 1994, 144: 1269-1280
Sedlacek R, Mauch S, Kolb B, Schatzlein C, Eibel H, Peter HH, Schmitt J, Krawinkel U: Matrix metalloproteinase MMP-19 (RASI-1) is expressed on the surface of activated periferal blood cells and is detected as an autoantigen in rheumatoid arthritis. Immunobiol 1998, 198: 408-423

Seiki M: Membrane-type matrix metalloproteinases. APMIS 1999, 107: 137-143

Shapiro S, Kobayashi D, Ley TJ: Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. J Biol Chem 1993, 268:23824-23829

Shapiro SD: Matrix metalloproteinase degradation of extracellular matrix: biological consequences. Curr Opin Cell Biol 1998, 10: 602-608

Shimada T, Nakamura H, Ohuchi E, Fujii Y, Murakami Y, Sato H, Seiki M, Okada Y: Characterization of a truncated form of human membrane type 3 matrix metalloproteinase. Eur J Biochem 1999, 262: 907-914

Shipley M Wesselschmidt R, Kobayashi D, Ley T and Shapiro S: Metalloelastase is required for macrophage mediated proteolysis and matrix invasion in mice. Proc Natl Acad Sci USA 1996, 93: 3942-3946

Shofuda K, Yasumitsu H, Nishihashi A, Miki K, Miyazaki K: Expression of three membrane-type matrix metalloproteinases (MT-MMPs) in rat vascular smooth muscle cells and characterization of MT3-MMP with and without transmembrane domain. J Biol Chem 1997, 272: 9749-9754

Siméon A, Monier F, Emonard H, Gillery P, Birembaut P, Hornebeck W, Maquart F-X: Expression and activation of matrix metalloproteinases in wounds: modulation by the tripeptide-copper complex glycyl-l-histidyl-l-lysine-Cu^{2+}. J Invest Dermatol 1999, 112: 957-964

Singer JA, Clark RAF: Cutaneous wound healing. New Engl J Med 1999, 341: 738-746

Slavin J: The role of cytokines in wound healing. J Pathol 1996, 178: 5-10

Smith MR, Kung H-f, Durum SK, Colburn NH, Sun Y: TIMP-3 induces cell death by stabilizing TNF-α receptors on the surface of human colon carcinoma cells. Cytokine 1997, 9: 770-780

Sonnenberg A, Calafat J, Janssen H, Daams H, van der Raaij-Helmer LM, Falcioni R, Kennel SJ, Aplin JD, Baker J, Loizidou M, Garrod D: Integrin α6/β4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J Cell Biol 1991, 113: 907-917

Stanley JR, Tanaka T, Mueller S, Klaus-Kovtun V, Roop D: Isolation of cDNA for bullous pemphigoid antigen by use of patients autoantibodies. 1988, 82: 1864-1870

Steed DL: The role of growth factors in wound healing. Surg Clin North Am 1997, 77:575-585

Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK: α6β4 integrin heterodimer is a component of hemidesmosomes. Proc Natl Acad Sci USA 1990, 87: 8970-8974

Stetler-Stevenson WG, Berch N, Golde DW: Tissue inhibitor of metalloproteinase-2 (TIMP-2) has erythroid-potentiating activity. FEBS Lett 1992, 296: 231-234

Stolow MA, Bauzon DD, Li J, Sedgwick T, Liang VC-T, Sang QA, Shi Y-B: Identification and characterization of a novel collagenase in Xenopus laevis: possible roles during frog development. Mol Biol Cell 1996, 7: 1471-1483

Stricklin GP, Li L, Jancic V, Wenczak BA, Nanney LB: Localization of mRNAs representing collagenase and TIMP in sections of healing human burn wounds. Am J Pathol 1993, 143: 1657-1666

Stricklin GP, Nanney LB: Immunolocalization of collagenase and TIMP in healing human burn wounds. J Invest Dermatol 1994a, 103: 488-492

Stricklin GP, Li L, Nanney LB: Localization of mRNAs representing interstitial collagenase, 72 kDa gelatinase, and TIMP in healing porcine burn wounds. J Invest Dermatol 1994b, 103: 352-358,

Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI: Mechanism of cell surface activation of 72 kDa type IV collagenase. Isolation of the activated form of the membrane metalloproteinase. J Biol Chem 1995, 270: 5331-5338

Sudbeck BD, Jeffrey JJ, Welgus HG, Mecham RP, McCourt D, Parks WC: Purification and characterization of bovine interstitial collagenase and tissue inhibitor of metalloproteinases. Arch Biochem Biophys 1992, 293: 370-376

Sudbeck BD, Pilcher BK, Pentland AP, Parks WC: Modulation of intracellular calcium levels inhibits secretion of collagenase 1 by migrating keratinocytes. Mol Biol Cell 1997, 8: 811-824

Suzuki K, Enghild JJ, Morodomi T, Salvesen G, Nagase H: Mechanisms of activation of tissue procollagenase by matrix metalloproteinase-3 (stromelysin-1). Biochemistry 1990, 10261-10270

Takino T, Sato H, Shinagawa A, Seiki M: Identification of the second membrane-type matrix metalloproteinase (MT-MMP-2) gene from a human placenta cDNA library. J Biol Chem 1995, 270: 23013-23020

Tani T, Karttunen T, Kiviluoto T, Kivilaakso E, Burgeson R, Sipponen P, Virtanen I: α6β4 integrin and newly deposited laminin-1 and laminin-5 form the adhesion mechanism of gastric carcinoma Continuous expression of laminins but not that of collagen VII is preserved in invasive parts of the carcinomas: implications for acquisition of the invading phenotype. Am J Pathol 1996, 149: 781-793

Thomas PF: Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 1980, 77: 5201-5205

Tremble PM, Lane TF, Sage EH, Werb Z: SPARC, a secreted protein associated with morphogenesis and tissue remodeling, induces expression of metalloproteinases in fibroblasts through a novel extracellular matrix-dependent pathway. J Cell Biol 1993, 121: 1433-1444

Tsuboi R, Sato C, Kurita Y, Ron D, Rubin JS, Ogawa H: Keratinocyte growth factor (FGF-7) stimulates migration and plasminogen activator activity of normal human keratinocytes. J Invest Dermatol 1993, 101: 49-53

Uitto J, Mauviel A, McGrath J: The dermal-epidermal basement membrane zone in cutaneous wound healing. In The molecular and cellular biology of wound repair. Ed. Clark RAF. Plenum Press, New York, 1995 pp. 513-559

Uitto V-J, Airola K, Vaalamo M, Johansson N, Putnins EE, Firth JD, Salonen J, López-Otín C, Saarialho-Kere U, Kähäri V-M: Collagenase-3 (MMP-13) expression is induced in oral mucosal epithelium during chronic inflammation. Am J Pathol 1998, 152: 1489-1499

Uria JA, Ferrando AA, Velasco G, Freije JP and Lopez-Otin C: Structure and expression in breast tumors of human TIMP-3, a new member of the metalloproteinase inhibitor family. Cancer Res 1994, 54: 2091-2094

Vaalamo M, Kariniemi A-L, Shapiro SD, Saarialho-Kere U: Enhanced expression of human metalloelastase (MMP-12) in cutaneous granulomas and macrophage migration. J Invest Dermatol 1999, 112: 499-505

Van der Rest M, Garrone R: Collagen family of proteins. FASEB J 1991, 5: 2814-2823

Van Wart HE, Birkedal-Hansen H: The cysteine switch: A principle of regulation matrix metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci USA 1990, 87: 5578-5582

Varedi M, Tredget EE, Scott PG, Shen YJ, Ghahary A: Alteration in cell morphology triggeres transforming growth factor-β1, collagenase, and tissue inhibitor of metalloproteinases-1 expression in normal and hypertrophic scar fibroblasts. J Invest Dermatol 1995, 104: 118-123

Velasco G, Péndas AM, Fueyo A, Knäuper V, Murphy G, López-Otín C: Cloning and characterization of human MMP-23, a new matrix metalloproteinase predominantly expressed in reproductive tissues and lacking conserved domains in other family members. J Biol Chem 1999, 274: 4570-4576

Virtanen I, Tani T, Bäck N, Häppölä O, Laitinen L, Kiviluoto T, Salo J, Burgeson RE, Lehto V-P, Kivilaakso E: Differential expression of laminin chains and their integrin receptors in human gastric mucosa. Am J Pathol 1995, 147: 1123-1132

von Boguslawski K: Immunohistochemical detection of progesterone in paraffin sections. APMIS 1994, 102: 641-646

von Bredow DC, Nagle RB, Bowden GT, Cress AE: Cleavage of β4 integrin by matrilysin. 1997, Exp Cell Res 236: 341-345

Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z: MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell 1998, 93: 411-422

Wang M, Liu YE, Greene J, Sheng S, Fuchs A, Rosen EM, Shi YE: Inhibition of tumor growth and metastasis of human breast cancer cells transfected with tissue inhibitor of metalloproteinase-4. Oncogene 1997, 14: 2767-2774

Wang Y, Johnson AR, Ye QZ, Dyer RD: Catalytic activities and substrate specificity of the human membrane type 4 matrix metalloproteinase catalytic domain. J Biol Chem 1999, 274: 33043-33049

Weckroth M, Vaheri A, Lauharanta J, Sorsa T, Konttinen YT: Matrix metalloproteinases, gelatinase and collagenase, in chronic leg ulcers. J Invest Dermatol 1996, 106: 1119-1124

Welgus HG, Jeffrey JJ, Eisen AZ: The collagen substrate specificity of human skin fibroblast collagenase. J Biol Chem. 1981, 256: 9511-9515

Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH: Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. J Cell Biol 1989, 109: 877-889

Werner S, Peters KG, Longaker MT, Fuller-Pace F, Banda MJ, Williams LT: Large induction of keratinocyte growth factor expression in the dermis during wound healing. Proc Natl Acad Sci USA 1992, 89: 6896-6900

Westermarck J, Kähäri V-M: Regulation of matrix metalloproteinase expression in tumor invasion. FASEB J 1999, 13: 781-792

Whitelock JM, Murdoch AD, Iozzo RV, Underwood PA: The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases. J Biol Chem 1996, 271: 10079-86

Wick M, Burger C, Brusselbach S, Lucibello FC and Muller R: A novel member of human tissue inhibitor of metalloproteinases (TIMP) gene family is regulated during G1 progression, mitogenic stimulation, differentiation, and senesence. J Biol Chem 1994, 269: 18953-18960

Wight TN, Heinegård DK, Hascall VC: Proteoglycans. Structure and Function. In Cell biology of extracellular matrix. Ed. Elisabeth D. Hay. Plenum Press, New York 1991, pp. 45-78

Wilhelm SM, Collier IE, Kronberger A, Eisen AZ, Marmer BL, Grant GA, Bauer EA, Goldberg GI: Human skin fibroblast stromelysin: structure, glycosylation, substrate specificity, and differential expression in normal and tumorigenic cells. Proc Natl Acad Sci USA 1987, 84: 6725-6729

Will H, Hinzmann B: cDNA sequence and mRNA tissue distribution of a novel human matrix metalloproteinase with a potential transmembrane segment. Eur J Biochem 1995, 231: 602-608

Wilson AJ, Gibson PR: Epithelial migration in the colon: filling in the gaps. Clin Sci 1997, 93: 97-108

Wilson CL, Heppner KJ, Rudolph LA, Matrisian LM: The metalloproteinase matrilysin is preferentially expressed by epithelial cells in a tissue-restricted pattern in the mouse. Mol Biol Cell 1995, 6: 851-869

Wilson CL, Heppner KJ, Labosky PA, Hogan BLM, Matrisian LM: Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc Natl Acad Sci USA 1997, 94: 1402-1407

Wilson CL, Matrisian LM: Matrilysin. In Matrix metalloproteinases. Eds. Parks WC, Mecham RP. Academic Press, Inc., San Diego 1998 pp. 149-184

Wilson Cl, Ouellette Ja, Satchell DP, Ayabe T, Lopez-Boado YS, Stratman JL, Hultgren SJ, Matrisian LM, Parks WC: Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science 1999, 286: 113-117

Windsor LJ, Grenett H, Birkedal-Hansen B, Bodden MK, Englers JA, Birkedal-Hansen H: Cell-type specific regulation of SL-1 and SL-2 genes. J Biol Chem 1993, 268: 17341-17347

Witte MB, Barbul A: General principles of wound healing. Surg Clin North America 1997, 77: 509-528

Witte MB, Thornton FJ, Kiyama T, Efron DT, Schulz GS, Moldaver LL, Barbul A: Metalloproteinase inhibitors and wound healing: A novel enhancer of wound strength. Surgery 1998, 124: 464-470

Woessner JF Jr: Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 1991, 5: 2145-2154

Woessner JF Jr: The matrix metalloproteinase family. In Matrix metalloproteinases. Eds. Parks WC, Mecham RP. Academic Press, California 1998, pp. 1-14

Wolf C, Chenard M-P, de Grossouvre D, Bellocq J-P, Chambon P, Basset P: Breast-cancer-associated stromelysin-3 gene is expressed in basal cell carcinoma and during cutaneous wound haeling. J Invest Dermatol 1992, 99: 870-872

Woodley DT: Reepithelialization. In The molecular and cellular biology of wound repair. Ed. Clark RAF. Plenum Press, New York 1996, pp. 339-354
Wysocki AB, Staiano-Coico L, Grinnell F: Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. J Invest Dermatol 1993, 101: 64-68

Yamada KM: Fibronectin and other cell interactive glycoproteins. In Cell biology of extracellular matrix. Ed. Elisabeth D. Hay. Plenum Press, New York 1991, pp. 111-146

Yamamoto H, Itoh F, Hinoda Y, Senota A, Yoshimoto M, Nakamura H, Imai K and Yachi A: Expression of matrilysin mRNA in colorectal adenomas and its induction by truncated fibronectin. Biochem Biophys Res Comm 1994, 201: 657-664

Yancey KB: Adhesion molecules. II: Interactions of keratinocytes with epidermal basement membrane. J Invest Dermatol 1995, 104: 1008-1014

Ye HQ, Azar DT: Expression of gelatinases A and B, and TIMPs 1 and 2 during corneal wound healing. Invest Ophtalmol Vis Sci 1998, 39: 913-921

Young PK, Grinnell F: Metalloproteinase activation cascade after burn injury: A longitudinal analysis of the human wound environment. J Invest Dermato. 1994, 103: 660-664

Yoshiji H, Buck TB, Harris SR, Ritter LM, Lindsay CK, Thorgeisson UP: Stimulatory effect of endogenous tissue inhibitor of metalloproteinases-1 (TIMP-1) overexpression on type IV collagen and laminin gene expression in rat mammary carcinoma cells. Biochem Biophys Res Comm 1998, 247: 605-609

Yurchenko PD, Schnitty JC: Molecular architecture of basement membranes. FASEB J 1990, 4: 1577-1590

Yurchenko PD, O´Rear JJ: Basal lamina assembly. Curr Opin Cell Biol 1994, 6: 674-681

Zhang K, Kramer RH: Laminin-5 deposition promotes keratinocyte motility. Exp Cell Res 1996, 227: 398-322

Ågren MS, Taplin CJ, Woessner F Jr, Eaglstein WH, Mertz PM: Collagenase in wound healing: Effect of wound age and type. J Invest Dermatol 1992, 99: 709-714

Ågren MS: Gelatinase activity during wound healing. Br J Dermatol 1994, 131: 634-640